CLINICAL PHARMACOLOGY and THERAPEUTICS

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volume 2 *number* 1 January-February 1961

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1. Taheri, Z. E.: Urevert in Cranial Trauma and Brain Surgery, J. Internatl. College of Surgeons *32*:389 (Oct.) 1959.
2. Javid, M.: Urea—New Use of an Old Agent, Reduction of Intracranial and Intraocular Pressure, The Surgical Clinics of North America, Philadelphia, W. B. Saunders Company, Aug. 1958, p. 907.

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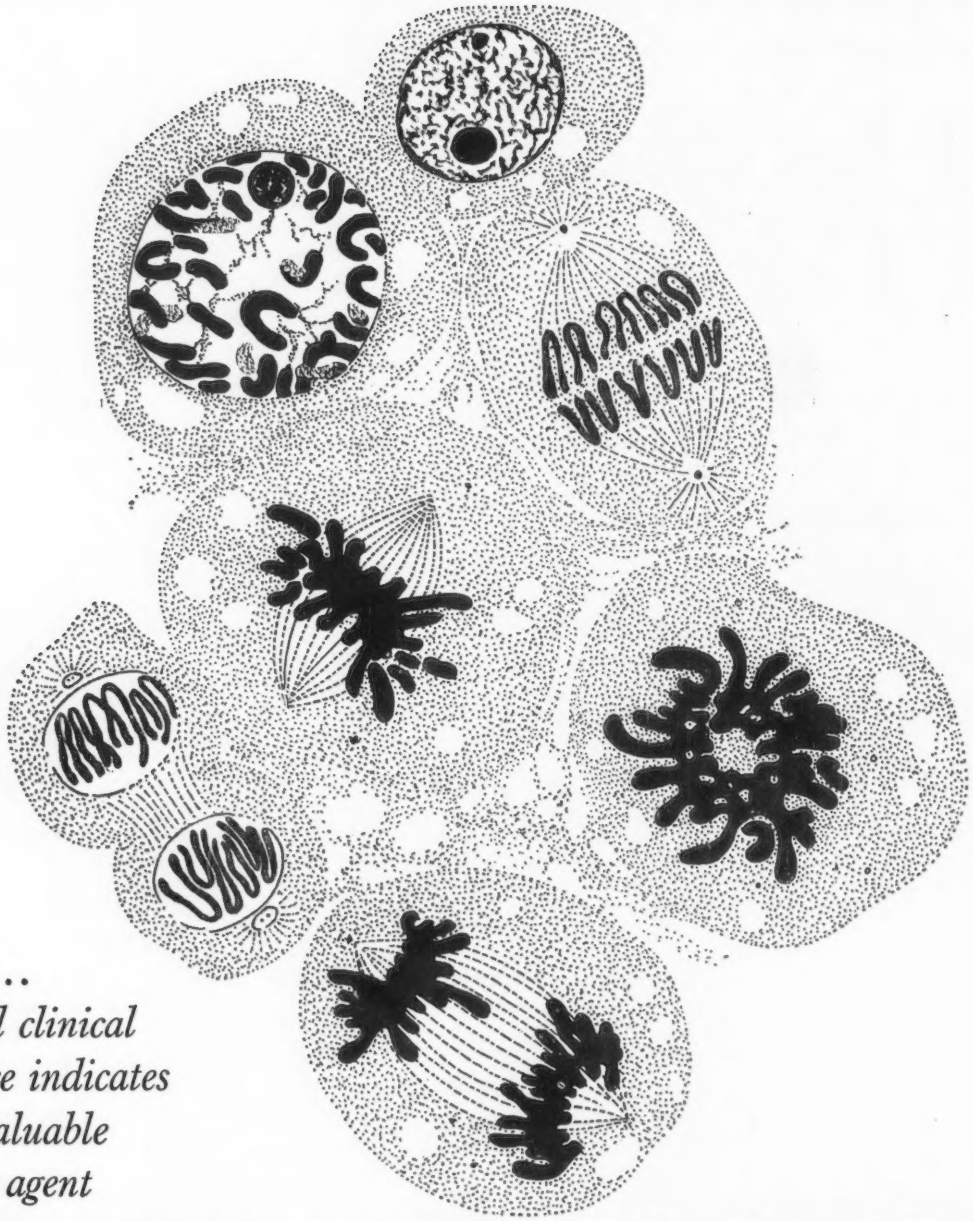

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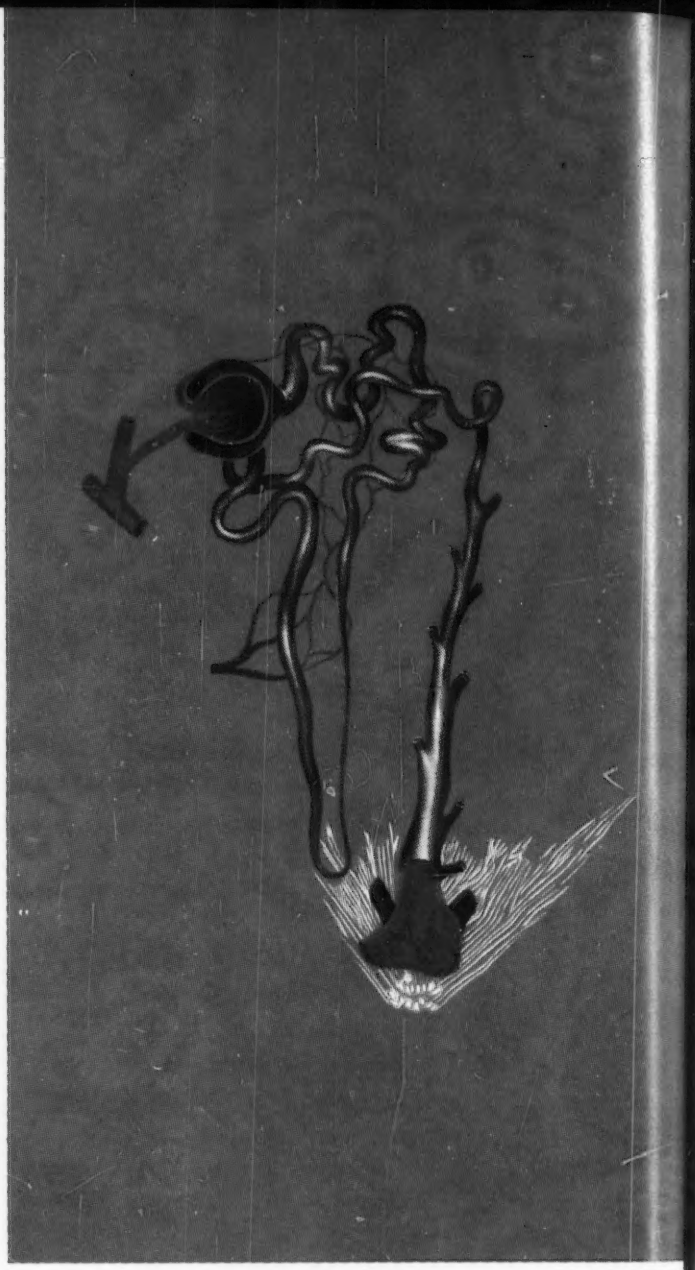

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References: 1. Schreiner, G. E.: A.M.A. Arch. Int. M. **102**:32, 1958. 2. Freedman, L. R., and Beeson, P. B.: Yale J. Biol. & Med. **30**:406, 1958. 3. Rocha, H., et al.: Yale J. Biol. & Med. **30**:341, 1958.

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articles to appear in early issues

Circulatory actions of general anesthetic agents and the homeostatic roles of epinephrine and norepinephrine in man

Henry L. Price, M.D., Philadelphia, Pa.

The effect of psychopharmacologic agents on behavior, measured by the interaction chronograph

Vicente B. Tuason, M.D., and Samuel B. Guze, M.D., St. Louis, Mo.

Anesthetic time/dose curves. III. The interaction of thiopental and oxymorphone during surgical anesthesia

M. Keeri-Szanto, M.D., M. Knaff, M.D., and Y. Rondeau, M.D., Montreal, Que.

Clinical pharmacology in industrial medicine

Leon J. Warshaw, M.D., F.A.C.P., New York, N. Y.

Factors affecting the absorption and biliary excretion of erythromycin and two of its derivatives in humans

James B. Hammond, M.D., and R. S. Griffith, M.D., Indianapolis, Ind.

The hypotensive effect of imipramine hydrochloride in patients with cardiovascular disease

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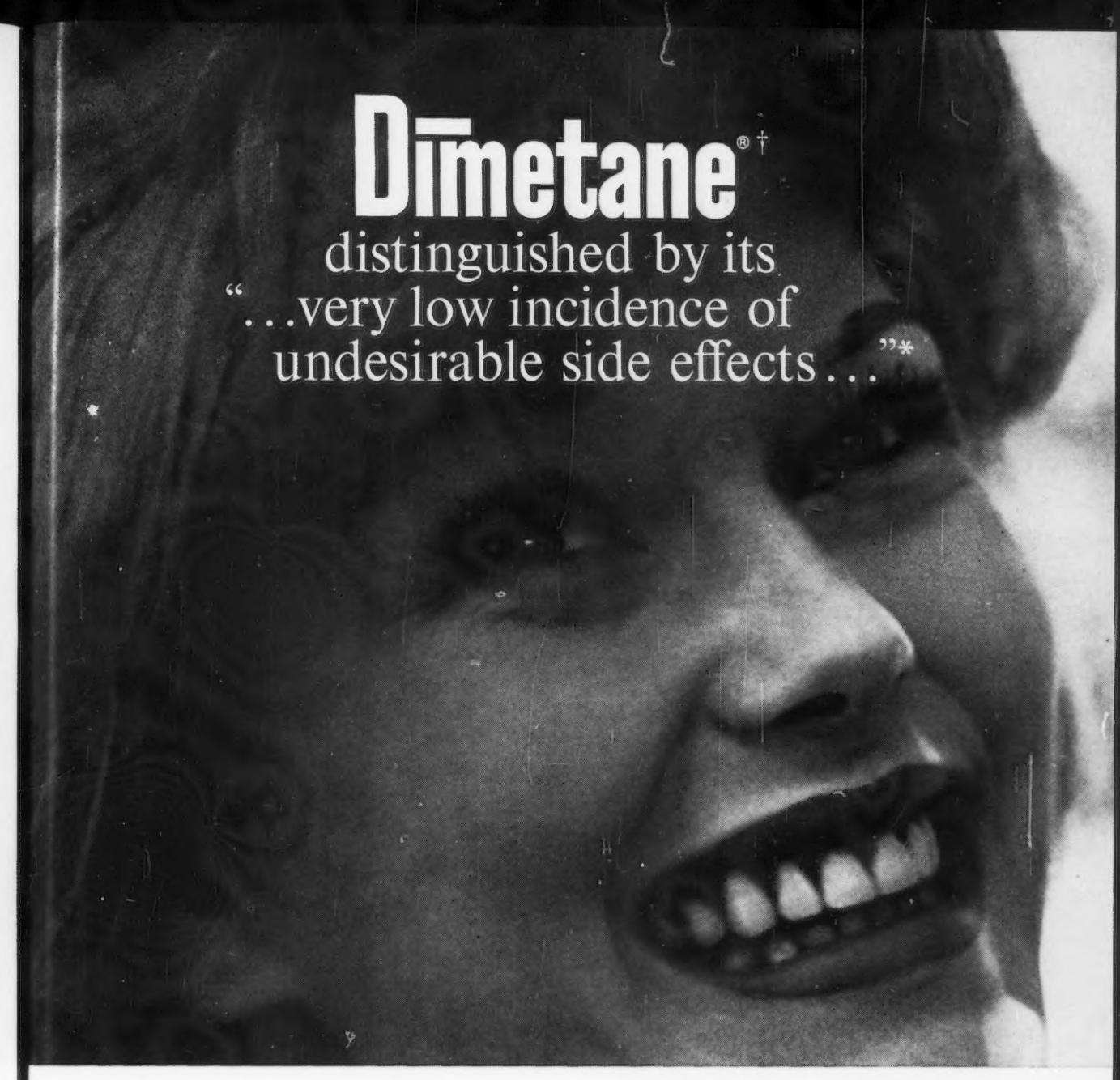
Symposium on the experimental pharmacology and clinical use of antimetabolites. Part VI. Mechanisms of resistance to metabolite analogues with anticancer activity

R. W. Brockman, Ph.D., Birmingham, Ala.



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Editorial

The drug explosion

If the pharmaceutical chemists took the time to look back at the net result of their prolificacy, would they be shocked to discover that the point of no return may have been passed? Do they suspect that now, instead of helping mankind with new drugs, they may be making matters worse? Will they realize that there is such a thing as too many drugs, that as matters stand there are too many drugs for the patient, for the physician, and, surprisingly enough, for the pharmaceutical industry? Although no one would suggest that they cease or even slow the pace of their search for useful drugs, if they are at all interested, I suggest that they do take the time to follow the effect of their creativity to its ultimate conclusion.

Five years ago, in an article entitled "Hazards of Modern Diagnosis and Therapy—The Price We Pay," Dr. David P. Barr pointed out that to the already staggering total of about 140,000 medicaments in current use, of which an estimated 90 per cent did not exist 25 years previously and 75 per cent had been introduced within 10 years, some 14,000 new ones had been added during the current year. Untoward reactions to medication have also increased at a staggering rate. This comes about primarily because of lack of experience with many different and entirely new active drugs and because of inability to master the full implications of these agents as rapidly as they are marketed and much less because of the unpredictable and unavoidable cases of hypersensitivity, which are relatively rare. Dr. Barr notes that on the medical service of one great hospital, 5 per cent of the patients—one of every 20—were admitted as the result of "sanctioned and well-intentioned" use of drugs. One needs only to read Moser's *Diseases of Medical Progress* (What an ironic title!) to realize the dimensions of this danger; Friend and Hoskins point out that there are now forty new diseases of this kind and that more are probably on the way.

As the number of new and active drugs increases and both knowledge and experience with each therefore become smaller and proportionately more difficult to obtain, not only is it a mathematic inevitability that drug reactions will also mount, but since more than a single factor is involved, it is also a certainty that the incidence of reactions will increase at an even faster rate than the rate at which new drugs

emerge. Even now the situation is alarming; but the future looks dismal indeed.

If this was all a hazard inherent in medical progress, in the well-intentioned search for better treatment for mankind, there would be some justification for it, but too often this is not the case. Too often new drugs are not introduced for the only proper reasons: because there is a real or presumed need for them, because they are genuinely superior to those in current use. Too often they are turned loose on the public to horn in on a market which has been created by someone else's discovery, to compete with drugs which have recently been established as good and useful. Too often they are hurried into use to get in on a market before it vanishes. I know of a pharmaceutical company in possession of a series of congeners which kept what it deemed to be the best for its own use and licensed the inferior ones to other distributors, to be sold by them to the medical profession for use on patients. Thus, drugs are being marketed and promoted and advertised by precisely the same techniques used for soaps and detergents.

A large proportion of claims for superiority for new drugs are patently invalid. But in addition, it is impossible that of the huge number of new drugs available, each one is the "best" for a separate medical indication. Thus for the approximately forty-five different tranquilizers, the thirty different sedatives, the eighteen different psychic energizers, the twenty-five different antihistamines, the thirty-two different antispasmodics, the thirty different diuretics on the market at this writing (and this census accounts only for different chemical entities and not different brands), each manufacturer claims his to be the best. Is each of the 150,000 preparations on the market the "best"? The best for what? The best for whom? There is a manufacturer who sells one drug entity in this country and a congener in another country, making precisely the same claims in each case: namely that each is the best for the same purpose. We are accustomed to this in soap advertisements, but drugs are not soaps. Since more than one drug cannot be the best for the same indication, we simply don't have enough diseases to go around. At the moment the most helpful contribution is the new drug to counteract the untoward effects of other new drugs; we now have several of these.

It is too bad that the American Medical Association gave up the publication of that small and masterful book, *Useful Drugs*. It provided a good, unbiased formulary for everyone. This, or its equivalent, is one way of ensuring both safe and effective use of drugs as well as limiting their number through authoritative suggestion. Is it the only way? Perhaps not, but how else clarify the confusion created by excessive numbers of unproved new drugs promiscuously and prematurely introduced into the drug market? Of course there would be no confusion if the pharmaceutical industry saw the immorality in claiming a drug to be the best when a better drug was in fact available, if the sole criterion for the introduction of a drug was the good of the patient.

The situation is more serious now than it was 5 years ago when Dr. Barr pointed out that untoward reactions to drugs "could be regarded as one of the commonest conditions encountered." This is understandable. There are now more drugs; some are extremely potent and exert diffuse effects, others interfere with basic physiologic function; many are most unusual pharmacologically, hence poorly understood; many suffer from limited clinical trial; all are vigorously advertised. And, it seems, too many are used with little discrimination. In commenting on this situation in a lecture, "The Rational Era of Therapeutics," Dr. K. J. R. Wightman stated that if ever we were in danger of irrational and irresponsible behavior as therapeutists, it is now.

Are physicians characteristically irrational and irresponsible? No! But they may sometimes appear to be because of the sheer impossibility of dealing rationally and responsibly with so many new drugs

about which so little is known but for which extravagant claims are made and for the use of which pressure is exerted by the drug industry and by patients who have heard of new cures through newspapers, magazines, and other patients. Vigorous drug promotion even before the drugs are available helps build up pressure to use them. It is beginning to look as if the success of a new drug will depend less on how well it works and more on how well it is promoted. This is why physicians are led to use drugs when the indications are lacking, to use drugs that are not the best available or even those which do not apply. It is because of this that the rate of serious drug reaction is mounting. And it is because physicians are not irresponsible that they may be expected to react with some violence to this ever mounting hazard.

That the situation gives every indication of worsening is suggested by the title of a paper on steroids, "How to Win at Structural Roulette." The pharmaceutical industry has had a prolonged winning streak at this game, but every winning streak ends some time. Already it is abundantly clear that the medical profession is one of the losers. It is gradually giving over its initiative in choosing drugs for its patients to the detail man because it cannot deal with the plethora of new drugs expertly, safely, effectively. Obviously, the public is an even heavier loser.

What will happen when, as it eventually must, physicians refuse to gamble with their patients' lives and health or an enraged public demands that such gambling stop? Certainly the winning streak of the pharmaceutical industry will come to an abrupt end, but the rebound may well be excessive and may lead to unhealthy cynicism on the part of physicians and a state of therapeutic nihilism.

If the pendulum then swings as far in the other direction, as pendulums do, the medical profession will tend to lean more and more on the handful of proved, established drugs such as morphine, penicillin, and digitalis, about which it can read substantial unbiased statements in textbooks, about which it will hear nothing from the detail man, and about which it will see nothing illustrated beautifully in drug house brochures, but on which it knows it can depend because of the accumulation of an enormous body of useful experience. And in this counterploy, surely important discoveries of our time will be overlooked and lost. How long before the public, medicine, *and the drug industry* are the losers to this type of general reaction?

What does the future hold if present practices continue? Twenty years ago the industry had already synthesized over 6,000 different sulfonamides; about a score or so were introduced and only about fifteen are in current use. There is no census on barbiturate synthesis, but although only about thirty were marketed in this country and about two dozen remain with us, the total number the chemists created certainly ran into many thousands. Several years ago one pharmaceutical company revealed that in its own laboratories it had synthesized and screened 1,000 nonphenothiazine tranquilizers. Thus there is a formidable stockpile of new and untried drugs which could be unloaded with very little notice. The present legal restraints could not effectively stop it, yet if all the active drugs available were introduced at one time, the result would be chaos.

In the past, the pharmaceutical industry has shown admirable restraint, but such restraint no longer exists. That drugs could be introduced still more rapidly is only a relative concession; it may be slow in relation to the rate which is possible, but otherwise it is far too rapid for the medical profession to acquire the knowledge essential for safe, effective use. Excessive numbers of drugs are now being introduced—excessive in view of the working capacities of those competent to test their safety and utility in man, excessive in view of the subjects available for the testing of their effects, dangers and uses in man, and excessive in view of the ability of those

who must assimilate the essential knowledge and learn how to prescribe them effectively and safely, rationally rather than routinely. This together with drug promotion and advertising, far more forceful than the comparative ignorance about them warrants, will lead just as inevitably to chaos, more insidiously perhaps than if all available new drugs were thrown on the market at one time, but the same chaos nonetheless.

If the therapeutic morbidity continues to rise, as it must under present conditions, it is clear that something will have to be done about it and this will be necessary if, as it gives every indication of doing, the pharmaceutical industry continues along its present lines. There is every reason to believe that the government will step in in the interests of public health unless some better and effective program is established first.

Is governmental control the only answer? Is it naive to hope that, as a few industries have in the past, the pharmaceutical industry would undertake to control its own practices? It seems to me that such an unusual procedure is justified because the pharmaceutical industry is an unique industry and it cannot operate in the same way or with the same attitude to its consumer public as other industries to. It must be concerned with the welfare of its public, of the public in general. It must have a high moral standard.

It makes little difference if, under the impression that it is the best, a housewife buys the next best detergent. *But you may not fool any of the people any of the time about drugs!* For even the slightest deviation from fact may be vital; if, under the misapprehension that it is the best, a doctor prescribes something less than the best, it may be the difference between life and death. Unlike the housewife and her detergent, it is clearly immoral if the physician is even *slightly misled* by claims made for the drugs he is importuned to use on the sick. It matters to him, it certainly matters to the patient, and it should matter to the pharmaceutical industry. There is the very real ethical question of whether the pharmaceutical industry has the right to sell all the drugs it creates and whether it does not have the moral obligation to select only the elite of its creations for use in man. There is no room for presumption or supposition. The catalog of the drug industry must be a "blue ribbon" list. If industry takes the view that as a purveyor of chemicals it can put all of its products on the open market, it should act as proper chemical manufacturers do and should remove itself from the field of drug promotion, certainly from a biased program of medical education.

I do not believe that the pharmaceutical industry is Public Enemy Number One or that its collective attitude is *après moi le déluge*. The industry has done many wonderful things for medicine and for mankind. It is to be hoped that it will continue to do so. But insofar as its operations are intimately connected with the life and health of the community, it has a moral obligation to the community which is in no wise lessened by its contributions of the past; perhaps the responsibility is even increased because of the precedents it has set. Unless it recognizes and acts on this aspect of its established function, does it not now stand in serious danger of having to give over its initiative as well as its controls to the government?

What can the pharmaceutical industry do? It seems to me that it can do a great deal. And it seems to me that it can do it more efficiently through its own devices than through any other agency, academic or governmental. Industry should undertake to control its practices. This would not be quixotic but, in my opinion, genuinely practical, really realistic. It should plan broadly for the effective screening of the drugs it synthesizes and terminate the current practice of the hurried introduction of new drugs in order to establish a foothold on the market while leaving the real testing of drugs in the hands of practicing physicians with patients as unwitting sub-

jects. Industry should undertake to limit the number of congeners of a single drug on the drug market to some practical number, to the two or three or four shown to be the best in industry's own grand clinical trials. Such a system would ensure a uniform high standard of preliminary investigation of new drugs.

While today most companies conduct careful and thorough explorations of new drug actions and toxicity in the laboratory as well as careful preliminary trials in the hands of experts before the drugs are marketed or even distributed for trial in the hands of clinicians, there are a few who, at a considerable saving in dollars and, often more important to them, a saving in time, put new drugs into circulation with the flimsiest minimum of preparatory work in order to inch in quickly on markets established by others. This inequity and immorality would be ended. To do this fairly as well as effectively, industry must arrange for cross-licensing so that companies which are not the patent holders of the drugs selected for general use can also distribute them and, therefore, will not lose all if their congeners are not chosen.

If clinical trials were carried out on a grand scale with an entire group of drugs examined in a coordinated program at one time instead of the present short-sighted system in which closely related drugs are examined separately and are not adequately compared, the truth about the group as a whole as well as the relative merits of its members would emerge much more promptly. Not only would the therapeutic morbidity rate fall but, perhaps even more important, since only the best drugs in each group would be available, patients would not be deprived of the best drug for their illnesses. A half dozen well chosen antihistamines would serve everyone's needs far better than the present twenty-five. The market would not be cluttered with near duplications for which the most conflicting claims are made. There would be more knowledge and more knowledgeable medical discussion and far less huckesterish mumbo jumbo about drugs. The Tower of Babel of drug names would collapse.

Can there be any doubt that under such a system the public and the medical profession would benefit? What about the pharmaceutical industry and its stockholders? If the medical profession merely continues to use drugs when they are needed, it will obviously not prescribe less after the inauguration of such a system than it does now. It is possible that it might prescribe even more because it would feel more secure; physicians might exploit drugs more fully, using more effective dosage. There might well be fewer token prescriptions given because of patient demands for the latest in drug development. If the industry manufactures larger amounts of smaller numbers of stable drugs, production costs should fall. Furthermore, if there are fewer drugs, more brands of the same drugs rather than more different drug entities, the very costly system of detail men and the enormously expensive and elaborate brochures which routinely stuff our mail boxes and monotonously extol the virtues of their principals should take no more of the pharmaceutical manufacturer's dollar than they are really worth.

Would not the discovery of new drugs pay off even better than now, since new drugs would be more firmly established by this system? Since their effective lives would be longer, their use through cross-licensing would be more extensive and the income to the discoverer through royalties should be greater. Would not the savings in drug production and distribution be enormous in such a system? Would there not be greater profits to industry as well as lower cost to the consumer? In this connection, it is suggestive that even now, of all the leaders in the field of pharmaceutical manufacture, one of the most successful (if not the most successful) of all American pharmaceutical companies from the point of view of gross profits introduces the smallest number of new drugs for a company of its magnitude and indulges in

virtually no molecule manipulation as the means of encroaching on markets established through the original research of other manufacturers. Those who follow such a system may therefore have larger profits as well as clearer corporate consciences. In addition, "the most ethical of the ethical companies" will not be forced by competition, as the *New England Journal of Medicine* points out they now are, to "meet the tactics of the least ethical."

It seems to me that the only segment of industry which may not wish to participate in an arrangement along the lines proposed because it cannot possibly profit by it is that small marginal portion which (1) has nothing to contribute to a cross-licensing system and (2) is interested only in seeing that its *own product,* rather than the *best medication* for the patient, is used in each instance. Since the organized industry will have gained such a high order of confidence of the medical profession and the public at large, should any firms refuse to participate, would they be able to compete with those who do? This system would serve to separate and distinguish in the *ethical* drug industry the ethical and the really ethical.

Because of coordination and increase in efficiency, the trials on humans could be more carefully and safely conducted. Because there would be far less waste of that invaluable commodity, the human subject, the clinical investigator would have much less trouble getting adequate clinical material for his needs and would be able to conduct more satisfactory clinical trials. Because this arrangement would eliminate much duplication and overlapping, that even rarer commodity, the expert clinical investigator, would be better able to deal with the heavy load provided for him by the new drugs. Finally, because clinical trials would be planned on a broad base, far less time and money should be wasted in preliminary trials before new drugs could be safely and properly marketed, and as a result there should be less delay in the safe use of new drugs.

It seems to me that advertising would continue at its present pace but would have to change from product promotion to institution promotion. Brand names would take on much more meaning because drugs would at once be identified with their distributor. The type of competition this would engender should lead to the highest standards in drug manufacture. It would no longer be essential for industry to educate (*sic*) the physician about drugs. Postgraduate medical education would return where it properly belongs, to the aegis of academic institutions, accredited medical journals, and medical societies.

Research in industry would continue at its present pace; new drugs will always be needed. But under such a system, those drugs which reach the market would be used without reluctance and cynicism because faith in industry's claims for drugs would be re-established. Since structural roulette would be restricted to the search for better drugs and not used to circumvent patent rights, research in industry would become far more meaningful than it presently is.

There can be no question: the medical profession's gain would be enormous. Because there would be fewer drugs in use, there would be a much larger over-all experience with each and more information about the unusual as well as the more common drug actions, about hypersensitivity, toxicity, synergism, incompatibility, and treatment of poisoning, of tolerance and addiction, and of use in refractory cases. Because the drug companies would be selling the same drugs, there would be few conflicting claims about their actions and effects, thus far less confusion. As the result of more knowledge and greater confidence, the physician would use his medicaments with greater assurance and exploit drugs to the greater good of his patients.

Will the patient benefit? How can the patient fail to benefit? Only the best drugs would be available. This is, of course, the most important consideration, but there are ancillary benefits as well. Because the phy-

sician would understand the drugs better and use them more effectively and with greater confidence, the patients would get better results from them. Certainly there would be far less iatrogenic disease. Finally, it seems likely that because the manufacturers of the best drugs would not be in terror of having them displaced through structural roulette or more effective promotion, they would not need to recoup their costs quite so quickly and could depend on a stable and prolonged market. And because there would be honest competition in the true American sense of the word, while drugs would cost the patient less, the profits of the manufacturer should also increase.

It could happen here.

Walter Modell, M.D.

Studies of analgesic drugs

VI. Comparative respiratory depressant activity of phenazocine and morphine

Phenazocine, the first benzomorphan compound to be used clinically, has been shown to be a potent analgesic in man. Early studies with this drug suggested that it possessed high analgesic potency, few respiratory or circulatory side effects, and low addiction liability

In this study, the relative respiratory depressant activity of equivalent analgesic doses of phenazocine and morphine were determined in normal adult males. Although there was significantly less respiratory depression 1 hour after phenazocine than after morphine, this difference was small and probably not of clinical significance. Respiratory depression persisted 3 hours after phenazocine, whereas morphine depression was waning at this time. On the basis of respiratory effects, peak action of phenazocine occurred between 30 and 90 minutes after intramuscular administration, and it persisted longer than morphine.

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Phenazocine, a synthetic potent analgesic, has aroused much interest recently because (1) it is the first of a new series of compounds, known as the benzomorphans, to be used clinically,[5, 7] (2) early data indicated that it was much less potent than morphine in suppressing the morphine abstinence syndrome of addicted monkeys, suggesting a lesser addiction liability than morphine,[3] and (3) early clinical experience with phenazocine indicated that it possessed high analgesic potency with little or no respiratory and circulatory effects.[4] For these reasons, further studies of phenazocine were undertaken in man, and this study was designed to compare the respiratory depressant activity of phenazocine with morphine.

Methods

Phenazocine is dl-2′-hydroxy-5,9-dimethyl-2-phenethyl-6,7-benzomorphan hydrobromide and has been referred to in other publications under code numbers NIH 7519 and SKF 6574. The first report of its analgesic effectiveness in an uncontrolled clinical trial suggested that phenazocine was 7 to 10 times as potent as mor-

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phine.[4] Subsequent controlled studies by Wallenstein, Rogers, and Houde[10] and by DeKornfeld and Lasagna[2] indicated that phenazocine was only 3 to 4 times as potent as morphine on the basis of weight of their respective salts. In this study, 2.5 mg. phenazocine hydrobromide per 70 Kg. was considered equianalgesic to 10 mg. morphine sulfate per 70 Kg.

The subjects of this study were 5 healthy men between the ages of 22 and 30 years (mean body area, 1.72 ± 0.12 sq. M.). Morphine and phenazocine were administered intramuscularly to all subjects in random order with a period of at least 1 week between successive trials. Subjects breathed through a mouthpiece attached to a nonrebreathing valve with a total dead space of 35 c.c. Expired gases were passed through a low resistance dry gas meter, by which expired minute volume and respiratory rate were measured. Alveolar air was sampled continuously by means of a Rahn end tidal alveolar air sampler and passed through an infrared carbon dioxide analyzer to determine alveolar carbon dioxide tension (P_ACO_2). Measurements were made at three intervals: before drug and 60 and 180 minutes after the drug. At each interval, the subjects breathed gas mixtures which approximated 2, 4, and 6 per cent of carbon dioxide in oxygen. Each subject breathed each gas mixture for 3 minutes to reach maximum ventilation before data were collected. Data were then collected during two 2 minute periods on each gas mixture, with 1 minute between consecutive collections. The mean values of these two periods provided the data for further analysis. Each run on all three gas mixtures required 25 to 30 minutes.

Alveolar ventilation (V_A) was calculated from expired minute volume corrected to 37° C. and respiratory rate, assuming a dead space of 150 c.c. The data of each subject at each time interval were plotted as a respiratory stimulus-response (P_ACO_2-V_A) curve, and the equation of the control curve was calculated. The slope of the control curve for each subject was then applied visually to the postdrug curves, and the displacement of the stimulus-response curve to the right at V_A 8.5 L. per minute was determined for each subject. This displacement represented in a single expression the degree of respiratory depression produced by the drug. The significance of the difference in mean displacements by the two drugs was determined by the *t* test for paired replicates.[8]

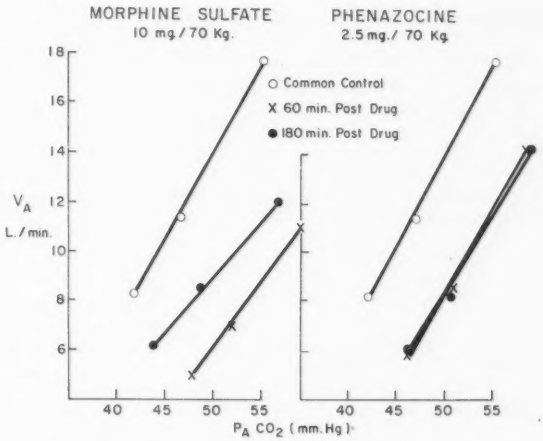

Fig. 1. Changes in respiratory stimulus-response curve after morphine and phenazocine as calculated from the total data. The common control (predrug) curve was obtained from ten measurements in 5 subjects. Postdrug curves were obtained from five measurements in 5 subjects. Abscissa: alveolar carbon dioxide tension; ordinate: alveolar ventilation.

Results

The mean data as collected are presented in Table I. From the total data, P_ACO_2-V_A curves were also calculated to investigate changes in slope. These curves are presented in Fig. 1. In this plot, the predrug curve for both drugs is the common control curve calculated from the ten control runs on the 5 subjects. This was considered the best estimate of predrug respiratory center sensitivity. Postdrug curves were calculated from five determinations, one on each subject. In this plot, the displacement of the respiratory stimulus-response curve 1 hour after morphine was greater than 1 hour after phenazocine.

Table I. *Effect of phenazocine and morphine on expired minute volumes (corrected to dioxide tension in 5 subjects breathing three concentrations of carbon dioxide in oxygen*

Time	2% CO_2 in O_2			
	Minute volume (L.)	*Respiratory rate (per min.)*	*P_ACO_2 (mm. Hg)*	*Minute volume (L.)*
Phenazocine (2.5 mg. per 70 Kg.)				
Control	10.90 ± 0.98	13.9 ± 0.8	42.2 ± 1.7	13.66 ± 1.53
60 min.	7.67 ± 0.54	12.6 ± 0.7	45.6 ± 1.8	10.02 ± 0.92
180 min.	7.73 ± 0.49	13.7 ± 0.9	45.8 ± 1.6	9.82 ± 0.56
Morphine (10 mg. per 70 Kg.)				
Control	9.54 ± 0.10	13.8 ± 1.3	41.1 ± 0.4	11.76 ± 0.37
60 min.	6.94 ± 0.30	12.3 ± 0.9	47.5 ± 0.7	8.94 ± 0.47
180 min.	8.14 ± 0.24	13.5 ± 0.7	43.7 ± 0.4	10.48 ± 0.36

Three hours after morphine, respiratory depression was waning whereas phenazocine depression persisted. With both drugs, the changes in the slope of postdrug curves were small. The slope of the common control curve was 0.56; the slopes 1 and 3 hours after morphine were 0.51 and 0.45; the slopes 1 and 3 hours after phenazocine were 0.40 and 0.51.

These differences between morphine and phenazocine were confirmed by comparison of the curve displacement for individual subjects when their predrug slopes were applied to the postdrug data (Table II). The mean displacement 1 hour after morphine was significantly greater than the displacement 1 hour after phenazocine ($P < 0.05$), but displacements were not significantly different 3 hours after the drug. Respiratory depression 1 hour after morphine was significantly greater than at 3 hours ($P < 0.05$), but there was no difference between the mean displacements at 1 and 3 hours after phenazocine.

Closer examination of the individual curve displacements by phenazocine (Table II) revealed that in 2 subjects respiratory depression was greater at 3 hours after drug than at 1 hour. In 2 other subjects, the displacement was only slightly less at 3 hours than at 1 hour. In only 1 subject (D. M.) was the depression of phenazocine waning significantly at 3 hours. Besides suggesting a longer duration of action with phenazocine, this observation raised the possibility that the peak action of phenazocine may have occurred between the 1 and 3 hours intervals selected for postdrug measurement. Since each run required 30 minutes, these measurements were actually made 60 to 90 minutes and 180 to 210 minutes after drug. The difference between the mean displacements by morphine and phenazocine could have resulted from a failure to measure phenazocine peak depression.

To investigate this possibility, 2 subjects were restudied on phenazocine in an identical manner with measurement made during the 30 to 60 minute, 90 to 120 minute, and 180 to 210 minute periods. These data together with those of the first trial are

Table II. *Displacement (mm. Hg) of respiratory stimulus-response (P_ACO_2-V_A) curves at V_A 8.5 L. per minute in 5 subjects after morphine and phenazocine*

Subject	*Morphine (10 mg. per 70 Kg.)*		*Phenazocine (2.5 mg. per 70 Kg.)*	
	60 min.	*180 min.*	*60 min.*	*180 min.*
D. M.	12.0	5.5	7.5	3.5
G. P.	11.6	5.5	10.7	9.8
J. H.	10.0	4.2	8.0	9.4
H. G.	11.5	6.0	6.9	5.8
L. R.	10.5	6.5	8.8	12.9
Mean	11.1	5.5	8.4	8.3

37° C.), respiratory rate, and alveolar carbon (mean ± standard error of mean)

4% CO_2 in O_2		6% CO_2 in O_2		
Respiratory rate (per min.)	*P_ACO_2 (mm. Hg)*	*Minute volume (L.)*	*Respiratory rate (per min.)*	*P_ACO_2 (mm. Hg)*
13.7 ± 0.6	48.3 ± 1.6	23.38 ± 2.03	15.9 ± 0.8	56.1 ± 2.0
13.2 ± 0.6	50.6 ± 1.6	15.62 ± 1.14	14.1 ± 0.6	58.1 ± 1.8
13.9 ± 0.4	51.5 ± 1.9	16.33 ± 1.16	15.5 ± 0.8	58.2 ± 1.3
14.9 ± 0.8	45.3 ± 0.5	15.62 ± 0.87	15.8 ± 0.8	53.2 ± 1.1
13.4 ± 0.8	51.9 ± 0.5	13.42 ± 0.63	15.0 ± 1.1	58.7 ± 0.4
13.8 ± 1.2	48.6 ± 0.8	14.34 ± 0.74	15.1 ± 1.0	56.4 ± 0.7

Table III. *Displacement of respiratory stimulus-response curves at several time intervals after phenazocine in 2 subjects who were studied on two occasions*

		Minutes after phenazocine (2.5 mg. per 70 Kg.)			
Subject	*Trial*	*30*	*60*	*90*	*180*
D. M.	1		7.5		3.5
	2	0		5.5	4.3
J. H.	1		8.0		9.4
	2	8.4		7.3	6.3

presented in Table III. In 1 of these subjects (D. M.), peak depression occurred during the 60 to 90 minute period; in the other (J. H.) peak depression was during the 30 to 60 minute period. In both, the depression was well sustained between the 90 and 180 minute observation periods. Thus, it was unlikely that the difference in mean displacements between phenazocine and morphine could be accounted for by differing peak effects.

During these studies, the subjects volunteered the following information concerning subjective effects of phenazocine. All subjects were sleepy, and 4 of the 5 experienced generalized itching. Two subjects complained of dizziness and 3 others of nausea without vomiting. All subjects were recumbent during the period of observation.

Discussion

From these data, there can be no doubt that phenazocine is a respiratory depressant. Although the depression 1 hour after phenazocine was less than after morphine, the clinical significance of this difference is questionable. The best estimates of the equianalgesic doses of these two drugs ranged from 2.5 to 3.3 mg. of phenazocine as equivalent to 10 mg. of morphine. The lower estimate was used in this study. Any difference between the drugs would have been less apparent with the higher estimate.

In general, our results agree with those of other investigators who have studied the respiratory depression of phenazocine specifically and disagree with those who observed little or no respiratory depression during clinical trials. In dogs, Stephen and Macmillan[9] found that 0.1 mg. phenazocine per kilogram depressed respiration as much as 5 mg. meperidine per kilogram. Translated to doses used in this study, the respiratory depression of 2.5 mg. of phenazocine was equivalent to that of 125 mg. of meperidine. Greisheimer and associates,[6] using normal subjects, could not distinguish between the respiratory depression of 2.5 mg. of phenazocine and 100 mg. of meperidine. Bellville and associates[1] studied several doses of both phenazocine and morphine and concluded that 1.7 mg. of phenazocine depressed respiration as much as 10 mg. of morphine.

The conclusion seems warranted that phenazocine is a respiratory depressant of approximately the same magnitude as morphine when given in equivalent analgesic doses. This provides another example of the seemingly obligatory relationship between analgesia and respiratory depression which has been demonstrated for almost all potent analgesics. Peak action of phenazocine occurred between 30 and 90 minutes after intramuscular administration, and its action was of longer duration than morphine.

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Metabolic effects and therapeutic applications of triiodothyropropionic acid

The effects of more than sixty analogues of thyroxine have been investigated in animals, and more than thirty analogues have been studied in humans. Animal studies have shown that structural alterations of the thyroid hormone cause not only large variations in potency but also dissociation of the various hormonal effects of thyroxine. Dissociation of thyroid hormonal effects has also been demonstrated in the human with triiodothyropropionic acid.

The drug is satisfactory as maintenance therapy in myxedema and appears to have definite advantages in maintaining the myxedematous patient with angina pectoris.

Triiodothyropropionic acid effects a 15 to 20 per cent drop in the serum cholesterol of about 80 per cent of euthyroid individuals with either elevated or normal cholesterol levels. Its administration in the proper dosage decreases elevated serum uric acid levels in both myxedematous and euthyroid individuals.

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In the past 5 years, more than sixty analogues of thyroxine have become available for comparison with thyroxine in experimental animals and for human use. Some thirty analogues have been tested in human subjects, and several have been tested in patients with diseases which might be benefited by a specific action of the thyroid hormone.

The thyroid analogues have been divided into several subgroups, based on the various chemical substitutions and changes in the basic thyroxine molecule, shown below. The positions in the thyroxine molecule occupied by the iodine adjacent to the phenolic hydroxyl group are the 3′,5′ posi-

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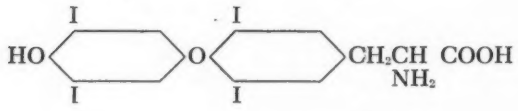
tions. The two remaining iodines occupy positions 3 and 5.



The natural hormones are levorotary, have an alanine side chain, and have iodine atoms in the 3,5,3′,5′ (thyroxine) position or in the 3,5,3′ (triiodothyronine) position. Since the carboxyl group on the alanine side chain is asymmetric, dextroisomers as well as levoisomers are possible, i.e., dextrotriiodothyronine, dextrothyroxine, etc. The amine group may be removed from the corresponding tetraiodinated or triiodinated natural compound to produce the propionic acid analogues. Shortening of the side chain or other alterations in the side chain give additional analogues. Iodine atoms may be removed from the phenolic rings, or, in the case of the triiodinated compounds, two iodines may be placed in the outer ring (or 3′,5′ positions), leaving a single iodine atom in the 3 or 5 position. Some of the latter compounds inhibit the action of thyroxine.[2, 12] Other atoms, or groups, i.e., halogens or NO_2, may be substituted for the iodine atoms. Finally, the ether connection between the two phenolic groups has been altered by substituting a sulfur atom, and groups have been substituted on the phenolic hydroxyl group.

Both animal and human studies have shown that dissociation of various hormonal actions can be effected by the chemical alteration of the phenolic rings and side chains of the basic thyroxine molecule. These alterations serve to enhance some actions of the hormone, while at the same time other actions are deemphasized.

In the present report, we describe preliminary results of a series of studies designed to establish the therapeutic effectiveness of one of the thyroid analogues, triiodothyropropionic acid, as a hypocholesterolemic agent in euthyroid individuals and as replacement therapy in myxedema complicated by angina pectoris.

Methods and materials

Studies on the therapeutic applications of triiodothyropropionic acid were performed on patients followed in the outpatient clinics of Memorial Hospital or of Bellevue Hospital. Patients were chosen from the following categories: (1) myxedematous patients, with and without angina pectoris, (2) euthyroid patients with nontoxic nodular goiters, and (3) euthyroid patients with hypercholesterolemia. The latter group was divided into three subgroups: patients with diabetes mellitus, patients with documented healed myocardial infarctions, with or without angina pectoris, and patients with uncomplicated hypercholesterolemia.

The diagnosis in the myxedematous group of patients was confirmed by determinations of serum protein bound iodine level and of thyroidal avidity for I^{131}. Some patients had previously been maintained on desiccated thyroid, l-thyroxine sodium, or liothyronine. Triiodothyropropionic acid

Table I. *Myxedematous patients maintained on triiodothyropropionic acid**

Patient	Sex	Dose (mg. per day)	Total serum cholesterol (mg. per 100 ml.)		Basal metabolic rate	
			Before treatment	On recorded dose	Before treatment	On recorded dose
F. V.	M	1.5	430	250		
L. N.	F	2.0	250	190		
C. M.	F	3.0	499	245	−20	+ 2
J. E.	F	2.0	350	137	−20	− 3
F. G.	F	3.0	263	180	− 9	− 1
W. F.	F	2.0	177	147	−28	− 5
E. E.	F	1.5	325	177		− 7
M. R.	F	2.5	311	207	−12	+15
B. S.	F	2.0	305	218		
Average		2.2	323	195		

*All determinations represent averages of two or more determinations.

was given in increasing doses to these patients until one of three end points was reached, depending on the study: (1) there was a significant drop in serum cholesterol, (2) the patient became euthyroid clinically or by basal metabolic rate measurements, or (3) angina pectoris occurred or increased.

An attempt was also made to shrink the thyroid with triiodothyropropionic acid in patients with nontoxic nodular goiters. No trial was considered significant unless it lasted 3 months or more at a maximum dosage. Serum cholesterol levels were followed in these patients.

The euthyroid patients with hypercholesterolemia were studied for a period of 2 to 4 weeks as outpatients before any therapy was given. At least two total serum cholesterol and uric acid determinations were made during the control period. No change was made or suggested in the patients' diet. Patients were then given gradually increasing doses of the compound and were seen at monthly intervals. All serum cholesterol and serum uric acid determinations were made in duplicate. Doses of medication were changed without regard to the serum cholesterol or uric acid levels, which were unknown to the clinical observers until several weeks later. Patients reported here were on medication for at least 1 month, and some were studied for periods of up to 18 months. At each visit, vital signs were determined, and the patient was questioned as to incidence of angina pectoris, change in exercise tolerance, the use of nitroglycerin, symptoms of thyrotoxicity, and side effects.

Serum total cholesterol determinations were done by a modification of the Schoenheimer-Sperry digitonin precipitation method.[16] Serum uric acid values were determined by a modification of the method of Archibald.[5] Basal metabolic rates, when examined, were determined by the Benedict-Roth method.[15] Goiter sizes were estimated by palpation and by use of calibrated beads about the neck at the level of a fixed landmark.

Table II. *Myxedematous patients with angina who were maintained on triiodothyropropionic acid**

Patient	Tolerated maintenance dose (mg. per day)	Previous therapy: Tolerated desiccated thyroid (mg. per day)	Total serum cholesterol (mg. per 100 ml.)†		Improvement‡
			B	A	
Patients who had previous therapy					
N. P.	4.0	120	230	219	√
V. L.	1.5	30			
D. L.	1.0	15	268	190	√
L. A.	1.25	60	269	220	√
M. B.	1.5	60	328	230	√
J. L.	1.0	15	240	180	√
Average	1.7	50	267	208	
Patients who had no previous therapy					
E. W.	1.5		210	109	√
J. L.	2.0				√
J. S.	0.6				
P. M.	1.2		202	140	√
B. H.	3.0		252	170	√
L. P.	1.0		230	201	√
Average	1.5		223	155	

All patients considered here showed either no change in frequency or severity of angina or were improved while on triiodothyropropionic acid. Comparison is made either to anginal attacks while patient was on maximum tolerated dosage of desiccated thyroid or while patient was myxedematous.

*All numbers represent average values.

†The top half of column B shows levels while on desiccated thyroid, the bottom half levels in the myxedematous state; column A shows levels on triiodothyropropionic acid.

‡Patients checked showed improvement over previous therapy in signs or symptoms of myxedema while on triiodothyropropionic acid.

Results

Table I summarizes some of the effects of triiodothyropropionic acid in uncomplicated myxedema. The average daily maintenance dose of the drug is 2 to 2.5 mg., with a range of 1 to 4 mg. On this dose, basal metabolic rates are maintained within normal limits, serum cholesterol is depressed from pretreatment levels, and abnormally high serum uric acid levels decrease. Table II includes 10 patients with myxedema and angina pectoris and shows the maintenance dosages which were tolerated. The average tolerated dose of the

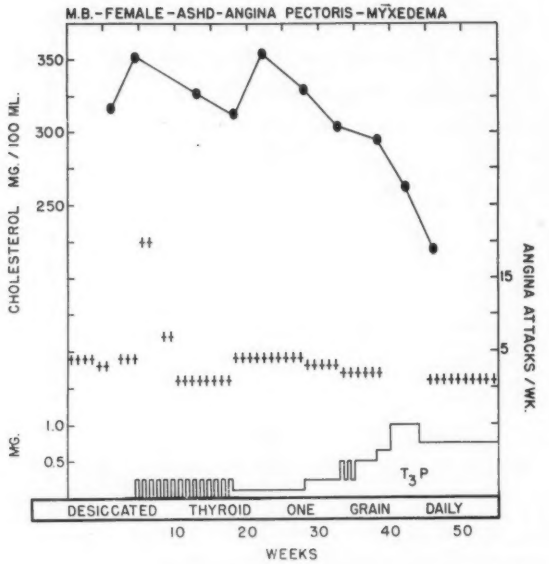


Fig. 1. The increased hypocholesterolemic effectiveness of triiodothyropropionic acid as compared to desiccated thyroid in a myxedematous patient with angina pectoris. On maximum tolerated doses of desiccated thyroid, the serum cholesterol was still well above normal limits. Adding triiodothyropropionic acid to the regimen lowered the serum cholesterol without causing an increased incidence of angina. The number of anginal attacks per week is represented by the crosses, with the scale on the right.

drug for this group was 1.5 mg. The important feature in this group is the decrease in serum cholesterol to normal values. This did not occur with desiccated thyroid in these patients.

Fig. 1 shows the course of 1 patient with myxedema and angina. When she took more than 60 mg. of desiccated thyroid daily, she had a marked increase in the number and severity of anginal attacks. While continuing to take 60 mg. of desiccated thyroid daily, triiodothyropropionic acid was added to the regimen in gradually increasing doses. Though the total serum cholesterol decreased significantly, there was no increase in the incidence or severity of anginal attacks during this period of observation.

Fig. 2 represents the average effect of triiodothyropropionic acid in 2 to 4 mg. daily doses on 25 euthyroid patients with hypercholesterolemia. Fourteen of these patients were men and 11 were women. Twelve patients, 8 men and 4 women, had hypercholesterolemia associated with diabetes. Two men and 3 women had had proved myocardial infarctions and 3 had had subsequent angina pectoris. Three men and 4 women had idiopathic hypercholesterolemia for miscellaneous reasons. One patient had nephrosis.

There was an average decrease in total serum cholesterol for the entire group of 15 per cent (45 mg. per 100 ml.) on 2 mg. per day, to 17 per cent on 4 mg. per day. The standard deviation in base line cholesterol levels for the group was 7.8 per cent, so that these changes are probably significant. Twenty of the 25 patients showed a significant drop on doses of triiodothyropropionic acid of 2 mg. or more. Four of the 5 who failed to show a significant depression of mean cholesterol were diabetics and had quite variable cholesterol values. The other patient who failed to show a significant decrease had the nephrotic syndrome. Some patients had sustained drops in serum cholesterol of 150 mg. or more.

Angina pectoris did not appear or become worse in any of these patients, and of the 3 who had had chronic angina, 1 patient reported a decrease in daily nitroglycerin intake from nine to three tablets on a maximum dose of triiodothyropropionic acid of 4 mg. daily. Insulin intake was decreased in 2 diabetic patients and increased in 1. One patient was taken off the compound (2 mg. per day) at a time when diabetes was out of control.

The average weight loss for the group (Fig. 2) was not significant in doses up to 4 mg. a day. Above that dose, weight loss did appear to be significant but was not related to other evidences of thyrotoxicity. The average pulse rates did not change on daily doses up to 4 mg.

An example of the hypocholesterolemic response is seen in Fig. 3. The serum cholesterol level varied directly with the changes in dosage. This individual was a 72-year-old female, with mild diabetes, who

previously had had a minor cerebrovascular accident. The patient was given up to 3 mg. a day. There was no significant change in pulse rate on any dosage given and no change in the diabetes.

That normal serum cholesterol levels can also be altered by administration of this compound is shown in Fig. 4. These patients all had nontoxic multinodular goiters. Serum protein bound iodine and I^{131} uptake were normal in all of this group, and the average total serum cholesterol level was 204 mg. per 100 ml. The cholesterol levels in this group were as sensitive, if not more so, than the levels in the hypercholesterolemic patients.

A somewhat unexpected observation in the group of 25 euthyroid patients was the apparent effect of triiodothyropropionic acid on the serum uric acid. Nine of 22 individuals had significant hyperuricemia. Three patients receiving chlorothiazide were omitted from the study. Of the 9 individuals, 2 were diabetic.

Table III lists the individuals with hyperuricemia and the results of the administration of triiodothyropropionic acid. When doses of 1 to 3 mg. per day were given to the individuals with hyperuricemia, there was a significant decrease in average serum uric acid values at each dose level in 8 of the 9. Fig. 5 shows graphically the results in the hyperuricemic group. The apparent rise of the uric acid on the higher dosages may or may not be real, since only 4 patients were studied at these dose levels, but individual patients exhibited the same pattern. Even individuals with normal, control uric acid levels tended to manifest slightly increased serum uric acid on the higher dosages.

Another possible therapeutic application of the thyroid analogues derived from the results of animal assays is their use as antigoitrogenic agents. We have given triiodothyropropionic acid to more than 40 patients with nontoxic multinodular goiters for periods of 3 months to over 1 year. Since the methods of measuring changes in goiter sizes are crude, it is difficult to be

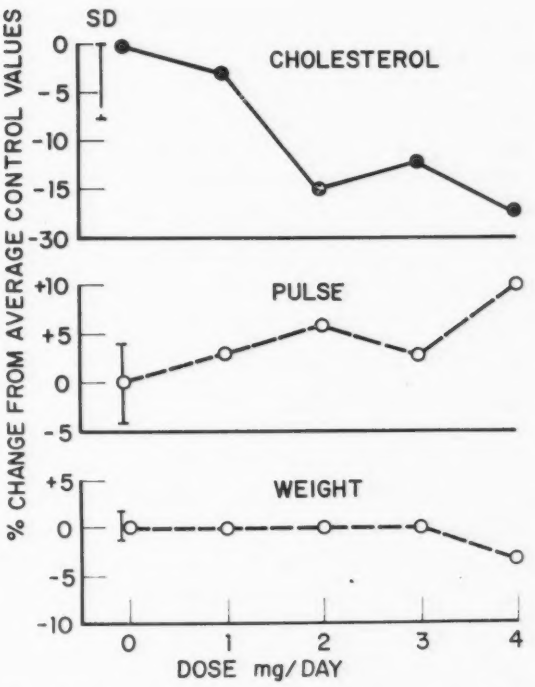


Fig. 2. The effect of triiodothyropropionic acid in 25 patients with hypercholesterolemia, representing average effect for total group. A 15 per cent change in serum cholesterol equals 45 mg. per 100 ml. A 5 per cent change in pulse rate equals 4 beats per minute. SD represents the individual standard deviations from the mean of pretreatment control values.

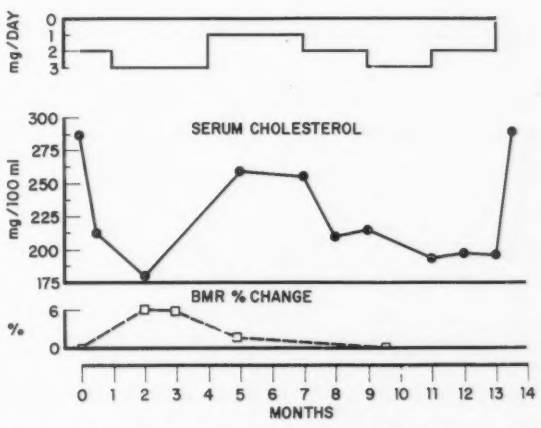


Fig. 3. The hypocholesterolemic effect of triiodothyropropionic acid in a euthyroid individual. The patient had mild chronic congestive cardiac failure but did not have or develop angina pectoris. Note the variation of serum cholesterol levels with the dose and the failure to cause a significant change in the basal metabolic rate.

precise, but somewhere between 25 and 50 per cent of goiters decreased in size on doses which did not induce excessive calorigenic effects.

Discussion and conclusions

It is obvious from the animal data[9, 10] that chemical alterations in the thyroxine molecule alter its over-all activity. It seems quite important, therefore, to study the effects of the sixty or more available analogues in the human.

In previous reports,[3-14] it has been shown that in the human, triiodothyropropionic acid, the analogue with which we have had the most experience, is probably rapidly absorbed and that it has only 2 to 10 per cent of the potency of l-thyroxine sodium on oxygen consumption. It has a biologic half-life in the serum of about 2 to 3 days and therefore is similar to other triiodinated compounds. The compound is about 50 per cent deiodinated within 24 hours and, like other thyroid analogues, leads to protein bound iodine values which are out of proportion to its activity. We have also presented evidence which suggests that it may have more of an effect on creatine and phosphorus excretion than does triiodothyronine in doses which cause equal increases in oxygen consumption.

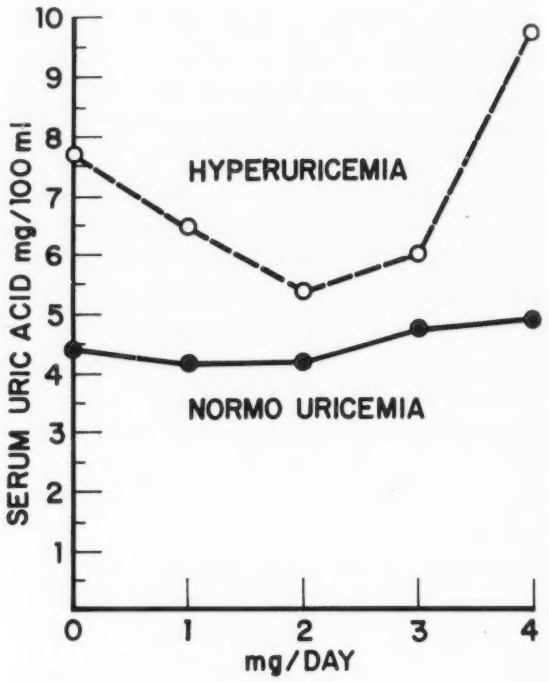


Fig. 5. The effect of triiodothyropropionic acid on uric acid in euthyroid individuals. Normal values are: men, 5.1 ± 1.4 mg. per 100 ml.; women, 4.1 ± 1.4 mg. per 100 ml.

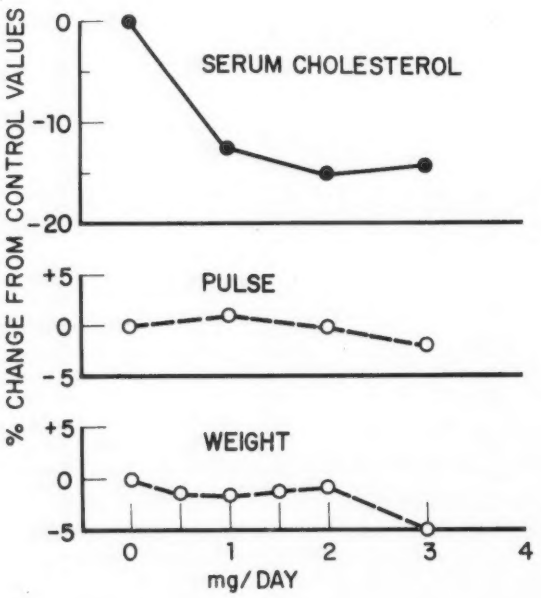


Fig. 4. The effect of triiodothyropropionic acid on normal serum cholesterol values. The average serum cholesterol value for this group was 204 mg. per 100 ml. Some reservations must be held concerning these data, since these patients all had nontoxic nodular goiters. However, all measurements of thyroid function were normal.

It is apparent that triiodothyropropionic acid,[3, 14] as well as other analogues, can cause depression of the serum cholesterol in myxedematous patients in doses which are not calorigenic. These doses average about 0.8 mg. daily for this compound. This dissociation of effects can also be demonstrated occasionally with small doses of thyroxine or triiodothyronine,[1, 7] but whether this separation is consistent and whether or not the difference between the hypocholesterolemic dose and the calorigenic dose is large is not known. We have calculated the ratio between the hypocholesterolemic dose and the calorigenic dose of triiodothyropropionic acid in myxedema to be about 1:4.

In myxedema, the average complete replacement maintenance doses is about 2.25 mg., with a range of about 1 to 4 mg. Patients have been well maintained on the drug for several months. Like triiodothyronine, the compound has the advantage of being rapidly metabolized and excreted. From our observations in myxedematous patients, we feel that it has less effect on the cardiovascular system than the natural hormones and may be especially useful in athyreotic patients with heart disease or angina pectoris. An important study which must be done should define whether or not the natural hormones have a specific action on the cardiac musculature which is not possessed by or is minimized in the various analogues. The recent work of Boyd and Oliver[4] would indicate that in fact some analogues do have less effect on heart rate and size than do the natural hormones. Although quantitation of the clinical condition is difficult, it appeared that the myxedematous patients with angina pectoris tolerated doses of triiodothyropropionic acid which caused more of a return to the euthyroid state than was the case with desiccated thyroid.

Studies in euthyroid patients have shown that thyroxine is capable of lowering the serum cholesterol in normal persons.[17] However, the associated calorigenic and cardiac effects of the natural hormones have been a deterrent to their use in patients with diabetes or cardiovascular disease.

A thyroid analogue the calorigenic potency of which is reduced compared to its hypocholesterolemic potency has obvious therapeutic possibilities. We have shown in this report that triiodothyropropionic acid, in doses which have little if any calorigenic effect, can be given in a clinical situation as a hypocholesterolemic agent. The percentage drop in cholesterols it causes is similar to that seen with many other agents, such as nicotinic acid or dietary management. The ease of administration of this compound has certain definite advantages.

The effect of the thyroid hormones on the serum uric acid in myxedema has only recently been described.[8] Since this was believed to be primarily a renal effect, we were somewhat surprised to see an apparent hypouricemic effect in euthyroid individuals. All of our patients who had hyperuricemia had normal or near normal blood urea nitrogen values. While it is realized that these are not good criteria

Table III. *Effect of triiodothyropropionic acid on hyperuricemia in euthyroid patients*

		Average serum uric acid levels (mg. per 100 ml.)						
			By dose (mg. per day)					
Patient	*Sex*	*Control**	*1*	*2*	*3*	*4*	*5 or more*	*Diagnosis*
1	M	6.6	7.5	7.3	6.5	6.5	8.0	Arteriosclerotic cardiovascular disease
2	M	9.1	5.1	4.7	4.9			Diabetes
3	M	7.4	6.7	4.6	5.3			Diabetes
4	M	11.3	7.7	6.6	8.4	12.4		Myocardial infarction
5	F	5.6	4.5	3.5				Myocardial infarction
6	M	6.5	6.0					Myocardial infarction
7	M	7.5	6.1	5.2	4.4	8.6	7.4	Hypercholesterolemia
8	M	9.1	8.6	7.3	7.9	8.3		Nephrosis, secondary gout
9	F	6.0		4.3	4.6			Hypercholesterolemia
Average		7.7	6.5	5.4	6.0	9.0		

*Normal serum uric acid (± 2 SD) values determined in 33 individuals in our laboratory are: women, 4.1 ± 1.4 mg. per 100 ml.; men, 5.1 ± 1.4 mg. per 100 ml.

of renal function, it is hard to believe that all these patients would have a markedly decreased renal blood flow. Gertler and White[6] have stated that people with atherosclerotic heart disease may have somewhat elevated uric acid, and it has been a clinical observation that hypercholesterolemia or hyperlipemia is often associated with moderate elevations of the serum uric acid.[11]

In any event, our preliminary data show that even in euthyroid individuals, triiodothyropropionic acid has a hypouricemic effect. The dose causing this effect, 1 to 3 mg., is about the same dose as effects hypouricemia in myxedema. This dose also approaches the usual maintenance dose of triiodothyropropionic acid in myxedema.

The few observations which suggest a hyperuricemic effect of larger doses must be confirmed. This rise in serum uric acid shown by our data may represent only biologic variability and thus negate all the data, or it may represent a rebound or escape phenomenon. Biphasic activity of the compound, however, could be possible. From studies now going on in our laboratory, it is suggested that the higher levels of uric acid seen at high dose levels may be due to an actual increase in purine turnover rates, although reversal of excretion patterns may also play a role.

A compound which will reduce goiter sizes serves two purposes. The desirability of reducing the goiter for cosmetic reasons is obvious; additionally, however, the reduction in size of a goiter may help in deciding whether or not it should be removed surgically. A goiter or nodule which is reduced markedly in size by therapy other than x-irradiation is also more likely to be noncancerous. Since the animal data suggest that triiodothyropropionic acid is more effective in preventing the thiouracil goiter than it is in elevating the basal metabolic rate, we chose this compound to study as an antigoitrogenic agent. Of all goiters, 25 to 50 per cent decreased in size, but the difficulty of getting exact measurements makes these data only suggestive.

A few conclusions can be made from these studies: Triiodothyropropionic acid can serve as replacement therapy in myxedema. In appropriate dosage, the compound may be more suitable in patients with angina pectoris or other cardiac problems than the natural hormones.

It can lower serum cholesterol levels in a large percentage of euthyroid patients in dosages which cause little or no calorigenic effects. This compound and possibly other thyroxine analogues may have hypouricemic effects at relatively low dosages. The significance and mechanism of this effect are being investigated.

A trial with triiodothyropropionic acid as a goiter-shrinking agent is indicated in patients with nontoxic nodular goiters.

Further and more definitive studies with these and other analogues must be performed.

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A comparative study of optimal medical care with and without azaserine in multiple myeloma

A comparative clinical study of optimal medical care plus azaserine versus optimal medical care plus a placebo was accomplished in 20 patients with multiple myeloma. Azaserine was originally studied in myeloma because of reported effects on a plasma cell neoplasm in mice. The controlled study was undertaken because suggestive activity in myeloma had previously been seen. The 9 azaserine-treated patients sustained more toxicity than the 11 placebo-treated patients. No important difference in tumor behavior was detectable between the azaserine- and placebo-treated groups as determined by physical examination or the several hematologic, biochemical, and roentgenologic measurements made.

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There is no remarkably successful technique by which effective compounds are chosen for chemotherapy of cancer in man. Many of the drugs in current use for a variety of tumors have initially been recognized because of effects in a few screening tumors: sarcoma 180, adenocarcinoma 755, and Walker carcinoma 256.

Another approach to screening is the choice of an experimental tumor with characteristics similar to a particular human tumor. In certain lymphocytic neoplasms of mice, susceptibility to a chemotherapeutic agent has predicted partial responsiveness of analogous human tumors.[3] We have attempted to apply this rationale to a plasma cell tumor of mice and multiple myeloma of man.

In 1954, Dunn[5] described a transplantable plasma cell neoplasm of the ileocecal

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region which originated 13 months after methylcholanthrene injection in a C3H mouse. Potter[9, 11] demonstrated that this neoplasm, 70429, was slow growing, and he converted it to a more rapidly lethal ascites tumor. When growing in the ascites form, the tumor cell no longer resembled a differentiated plasma cell. He found the ascites tumor highly susceptible to azaserine when administered in low doses for a prolonged period.

Azaserine is an antibiotic, originally isolated from a Streptomyces culture, which has since been synthesized.[2] It is moderately active against several bacterial species and fungi but not against viruses or protozoa.[6] It is partially effective against a battery of transplantable animal tumors.[4, 13, 14]

The drug has been explored in clinical use by Ellison and colleagues.[7] Some patients with Hodgkin's disease, chronic lymphocytic leukemia, and acute leukemia showed limited response to administration of the drug, but no change was noted in 21 other individuals with a variety of neoplasms.

The chemotherapy of multiple myeloma in man is not eminently successful. Search for better agents is reasonable.[1, 12] The possible usefulness of a plasma cell neoplasm in mice as a screening device for myeloma in man prompted an exploratory study of the effectiveness of azaserine in myeloma.* Five patients with myeloma were studied, of whom 1 showed slight decrease in abnormal plasma protein concentration and resolution of a large subcutaneous infiltration. This early suggestion of activity of the drug in myeloma served as basis for the present study.

Methods

Physicians in five institutions cooperated in the design, execution, and evaluation of the study.

For entry into the study, it was required that the diagnosis of multiple myeloma be unequivocal. Acceptable evidence consisted of a clinical syndrome compatible with the disease and 10 per cent or more plasma or myeloma cells in 1,000 marrow cells counted. In addition, a protein abnormality in serum or urine or a clearly delineated tumor mass or osteolytic lesion was required for purposes of measurement. Patients with concomitant diseases known to be associated with marrow plasmacytosis were excluded. Uremia (blood urea nitrogen greater than 30 mg. per 100 ml. or nonprotein nitrogen greater than 50 mg. per 100 ml.) prevented entry into the study. Thrombocytopenia and neutropenia probably from prior treatment also excluded patients from the study until these findings had reverted to at least 75,000 platelets and 1,000 neutrophils per cubic millimeter, respectively. Chemotherapy (except azaserine) or radiation treatment did not exclude patients from study if it had been given at least 21 days previously.

Patients were allocated randomly into two groups. One group was treated with a regimen of optimal medical care plus azaserine; the other group was treated with the same regimen of optimal medical care plus a placebo. The azaserine and placebo capsules were coded in one of six categories to avoid inadvertent code break, and neither patient nor investigator was informed of the nature of the medication until after evaluation of all cases had been completed by vote of the cooperating investigators. The technique of case evaluation has been described elsewhere.[15]

The concurrent use of steroids, urethane, extensive irradiation, or other experimental drugs was not allowed. One course of localized irradiation for intolerable pain was permitted during the course of study if necessary. Transfusions, antibiotics, and narcotics were used as required.

The experimental treatment agent was administered once daily orally after fasting. Sodium bicarbonate (approximately 2.5 Gm.) was ingested at the same time to prevent inactivation of azaserine by gastric

*J. F. Holland and W. Regelson: Unpublished observations.

acid. The schedule of drug dose was 3 mg. per kilogram per day for 28 days, 6 mg. per kilogram per day for the next 7 days, 9 mg. per killigram per day for the next 7 days, and 12 mg. per killigram per day for the last 7 days, unless toxicity appeared first. In instances of mild alimentary toxicity, the drug was continued without change in dose, whereas moderate or severe alimentary toxicity, hematologic depression, or "systemic intoxication" required interruption of drug administration and reinstitution at lower levels after the toxic manifestations cleared. If the protocol were followed without interruptions for toxicity, a patient would have received 273 mg. per kilogram in 49 days. Observations were continued for 14 days after cessation of drug.

The azaserine used came from a single synthetic batch and was stored identically in all institutions, in a desiccator in a refrigerator. At the end of the study, representative capsules were shipped to the manufacturer, who found no evidence of drug deterioration during the 2 years after manufacture.

Results

Twenty-two patients were selected for study. Upon review of the completed cases, however, 2 patients assigned to azaserine were found not to satisfy the criteria for admission to the study and have been dropped from further consideration.* In Table I, some characteristics of the patient population are presented. Although essentially the same in age and sex distribution,

Table I. *Characteristics of patients studied*

Data	*Azaserine group*	*Placebo group*
Total	9	11
Male	4	6
Female	5	5
Median age	60	61
Range	47-71	48-83

Table II. *Prior treatment for multiple myeloma among 20 patients*

Data	*Azaserine group*	*Placebo group*
Total patients	9	11
No prior treatment	4	3
Prior treatment	5	8
Urethane	5	2
X-ray	1	3
Corticosteroids	3	6

prior treatment experience differed somewhat for the two treatment groups (Table II). The azaserine group contained more individuals previously treated with urethane, while the placebo group contained more patients with prior exposure to corticosteroids.

At the time of initiation of the study, all patients were in frank relapse from any prior therapeutic response. Hematologic, biochemical, roentgenologic, and physical examination data at the outset of study are presented in Table III. No important difference is seen between the groups.

After the completion of the study, analysis of patient records for those data which might have influenced the votes of the group was performed. The data are presented in Table IV. No important differences are seen. Three placebo-treated patients received local radiotherapy to painful bone disease in the sacrum, lumbar spine, and hip, respectively; no azaserine-treated patient was given radiotherapy.

The results of voting are shown in Table V. Three azaserine-treated patients sustained findings interpreted as substantial

*A 65-year-old woman died of a pulmonary embolus 3 days after drug assignment but before she had taken any azaserine. A 48-year-old man with marrow plasmacytosis of 11 per cent 5 months earlier was assigned to azaserine when the marrow plasma cell count was only 3 per cent. He had no osteolytic disease or Bence-Jones proteinuria. His total protein concentration was 10 Gm. per 100 ml., of which 5.9 Gm. per 100 ml. was globulin. He had 1 + proteinuria and a normal nonprotein nitrogen concentration. Azaserine was administered for 7 of 9 weeks at 3 mg. per kilogram per day and for 1 of 9 weeks at 1.5 mg. per kilogram per day. Drug was omitted for 1 week when a lip ulcer appeared after the fourth week. Another ulcer appeared in the ninth week. There was no improvement in his clinical or chemical status.

Table III. *Hematologic, biochemical, roentgenologic, and physical evidence of multiple myeloma at the outset of study*

Evidence	*Azaserine group*	*Placebo group*
Marrow plasma or myeloma cells per 1,000 cells counted		
Median	305	380
Range	110-950	130-900
Leukocytes per cu. mm.		
Median	5,200	5,200
Range	3000-6700	2900-7900
Thrombocytes $\times 10^3$ per cu. mm.		
Median	252	226
Range	180-365	92-535
Total serum protein, Gm. per 100 ml.		
Median	8.9	10.5
Range	5.3-13.4	6.2-13.7
Bence-Jones proteinuria present/Total tested	1/9	3/11
Proteinuria present/Total tested	4/6	3/6
Osteolytic bone disease present/Total x-rayed	7/8	11/11
Subcutaneous tumor mass present/Total patients	1/9	1/11
Serum calcium concentration > 12 mg. per 100 ml./ Total tested	1/8	3/10

toxicity evidenced, respectively, by nausea, vomiting, hypercalcemia, increasing uremia, and worsening of bone disease; nausea, vomiting, and lethargy; and nausea, vomiting, oral ulcerations, and fainting. One placebo-treated patient developed findings interpreted as substantial toxicity evidenced by oral ulcerations and systemic toxicity. One patient in each group was voted by the majority to have some recognizable subjective improvement, and in both instances, this was slight. No patient evidenced sufficient subjective and objective improvement to be voted by the majority as an instance of benefit in total evaluation of the drug trial.

Life table analysis of survival from onset of treatment and from diagnosis shows no consistent difference between the two treatment groups (Fig. 1).

Toxicity, defined as need to alter drug schedule, was prominent in the azaserine-treated group (Table VI). Although only 3 patients were voted as having shown "major untoward effect of treatment" (Table V), it is notable that 7 patients of 9 sustained toxicity while taking azaserine.

Discussion

The premise that transplantable animal neoplasms constitute an effective screening tool for drugs of use in human cancer has been repeatedly questioned.[8, 13] In this study, an extrapolation was made to human multiple myeloma from plasma cell tumor 70429 of mice. Earlier suggestive response in one man,* was not seen in the patients observed in this study, and it appears that if azaserine has any activity in human myeloma, it is of a sufficiently low order of magnitude as not to have been detected in this group of patients. Another mouse plasma cell tumor recently described is more distinctly analogous to the human disease, e.g., osteolytic bone lesions and abnormal circulating globulins occur.[10] It is possible that knowledge of drug effects on this tumor might provide compounds for clinical trial with a better chance of clinical activity. Unfortunately, this information is not at hand.†

*J. F. Holland and W. Regelson: Unpublished observations.

†M. Potter: Personal communication, 1959.

Table IV. *Change in measurements during treatment with azaserine or placebo from values at onset in patients with multiple myeloma**

Measurement	Azaserine (9 patients)				Placebo (11 patients)			
	No information	*Increase*	*No change*	*Decrease*	*No information*	*Increase*	*No change*	*Decrease*
Marrow plasma or myeloma cell count	3	2	4	0	2	2	7	0
Osteolytic bone disease	0	4	5	0	0	4	7	0
Total serum protein	0	0	9	0	0	0	11	0
Abnormal serum globulin†	2	1	6	0	4	0	7	0
Bence-Jones proteinuria	0	1	8	0	0	1	9	1
Proteinuria	3	0	5	1	5	0	6	0
Leukocytes	0	1	5	3	0	1	7	3
Thrombocytes	0	0	6	3	0	0	11	0
Serum calcium	1	1	7	0	2	1	8	0
Serum phosphorus	0	1	7	1	0	1	9	1
Alkaline phosphatase	0	1	8	0	0	0	9	2
Blood urea nitrogen or nonprotein nitrogen	0	1	8	0	0	0	11	0

*Increase, decrease, and no change were determined by the senior author on inspection of serial values for each patient. The appraisal is subjective in that fluctuations within the usual range of laboratory error for the particular test were counted as no change and stress was laid on confirmation of a trend of values. Values which are abnormal in Table III and which remained statically abnormal are listed as no change. The appraisal is unbiased insofar as it was done before the medication code, dose, interruptions, or toxicity data were analyzed.

†"M" plus γ globulin, electrophoretically determined, except 1 patient for whom total globulin by chemical fractionation was used.

The design of the present study provided the opportunity to study concurrently drug effects and spontaneous variations in the placebo-treated group. There was an unequivocal absence of objective therapeutic effectiveness of azaserine in the 9 patients who received it.

Table V. *Therapeutic and toxic effects of drug administration*

Effect	*Azaserine*	*Placebo*
Total patients	9	11
Reduction in size of one or more tumor masses (physical examination, x-ray)	0	0
Major untoward effects of treatment*	3	1
Some benefit to patient*	1	1
Total evaluation: benefit to patient	0	0

*See text.

Whether one could recognize a compound with therapeutic activity in a series of 9 treated patients is, of course, dependent on the true level of effectiveness of the agent. A drug which is therapeutic in only 11 per cent of cases could readily be missed in 9 consecutive patients. The chance of nine consecutive failures for such a drug is 35 per cent. However, if the true therapeutic effect of the drug were 33 per cent, there would be only a 3 per cent chance of observing nine failures. Consequently, rejecting a drug after nine consecutive failures would appear to be a reasonable experimental technique when the level of interesting effectiveness of the drug is to be about 33 per cent. Of course, if one or more definite beneficial treatment effects had been observed, it would have been necessary to estimate the degree of effectiveness by studying more patients. There was no evidence of definite benefit resulting from placebo in this study. Such a group served to minimize possible investi-

gator bias and "new agent" optimism. Studies of this general design are in progress in our Group to evaluate urethane in treatment of myeloma with placebo as control.

The comparability of treatment groups was good (Tables I, II, and III). The relative predominance of prior urethane treatment in the azaserine group (5 of 9 versus 2 of 11) and of corticosteroids in the placebo group (6 of 11 versus 3 of 9) probably did not seriously influence the results of the study. No sustained beneficial effect from prior therapy was present in any patient at the start of this study.

No important tumor suppression occurred during the study (Tables IV and V). It is of note that eleven of the seventeen marrow specimens from both groups on which information was available at the end of study showed no significant change in number of plasma or myeloma cell elements from the count at onset of study.

The only instance voted to be of some benefit to a patient receiving azaserine was balanced by a similar circumstance in the placebo-treated group (Table VI). The improvement in each instance was slight and subjective in nature. It is not clear why 3 of 11 placebo-treated patients received radiotherapy for bone pain during the study period whereas none of 9 azaserine-treated patients did. Review of the clinical records does not suggest that azaserine has analgesic properties.

Table VI. *Toxicity while taking azaserine or a placebo*

Data	*Azaserine*	*Placebo*
Toxicity absent	2	10
Toxicity present	7	1
Nausea and vomiting	6	0
Mouth lesions	3	1
Systemic intoxication	3	1
Thrombocytopenia	1	0
Days to first downward modification of drug dose, median	8	*

*Median patient completed 49 day protocol without drug reduction.

Toxicity occurred with sufficient regularity in the azaserine group to vitiate in large part the blind nature of the drug. This situation did not seem to alter adherence to the experimental design but is an example of one of the difficulties in performing theoretically ideal experiments in the clinic. If the results of the study had warranted, antitumor response could have been judged by the group on clinical data sheets without information concerning toxicity.

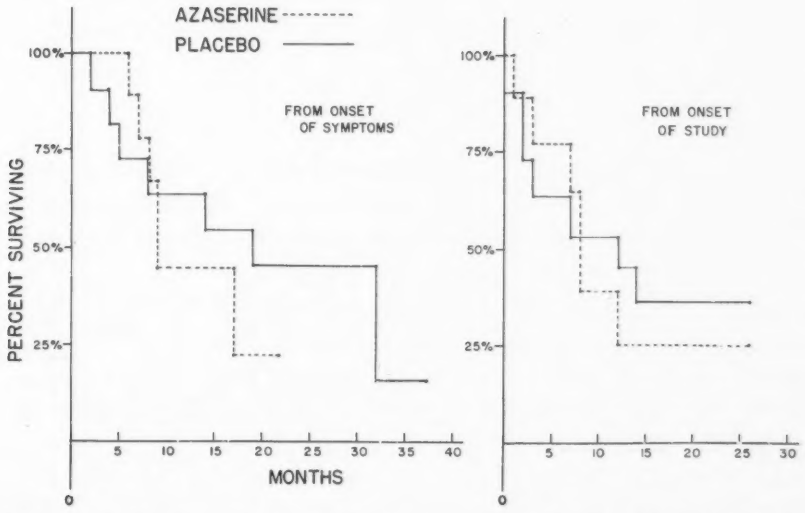

Fig. 1. Life table analysis of patients with multiple myeloma treated with optimal medical care plus azaserine or placebo.

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Demethylchlortetracycline

A clinical evaluation

Demethylchlortetracycline, a recently available member of the tetracycline group of antibiotics, has been evaluated clinically in 59 patients seen in a Veterans Administration Hospital with infections of the lower respiratory tract, urinary tract, or skin. The drug appeared to be comparable to other tetracyclines, and if the isolated etiologic organism was sensitive to DMCT in vitro, patients generally responded favorably. The study illustrated the great difficulty facing the physician who attempts to evaluate a new antimicrobial drug which closely resembles other good drugs. A majority of patients with infection suffered from underlying illnesses which predisposed them to infection or modified the infectious process through alteration of anatomic or physiologic host conditions. In this complex situation, the suppression of microbial growth—the sole effect of an antibiotic—contributes only to a variable and unpredictable extent to the relief of symptoms and signs of infection.

Among undesirable effects of oral DMCT administration were prominent gastrointestinal upsets with doses in excess of 600 mg. daily and a suggestion of renal and hepatic function impairment, which requires further study.

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A new tetracycline congener, demethylchlortetracycline, was first reported in 1957.[1] This antibiotic is produced by a mutant strain of *Streptomyces aureofaciens* and differs from the original chlortetracycline only by absence of a methyl group in the 6 position. Demethylchlortetracycline (DMCT) has been stated to produce "higher and better sustained" levels of antibacterial activity in the serum than corresponding doses of tetracycline, chlortetracycline, or oxytetracycline.[2-5] It was the purpose of the present study to evaluate the oral preparation of this drug clinically.

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Table I. *Patients with pulmonary infections treated with demethylchlortetracycline*

Case no.	*Diagnosis*	*Other diagnoses*	*Organism*	MIC*	*Date DMCT started*	*Fever to normal*	*Result*	*Comment*
2	Pneumonia, LLL	Emphysema; cor pulmonale	*D. pneumoniae*		Oct. 8, 1959	24 hours		Died March 20, 1960, of bronchopneumonia
18	Pneumonia, LLL	Malnutrition	*D. pneumoniae*	0.62	Jan. 13, 1960	7 days	Cured	Viable pneumococci isolated 6 days after therapy was begun
26	Pneumonia, RLL		*D. pneumoniae*	0.31	Oct. 19, 1959	7 days	Cured	Coin lesion RLL—undifferentiated; carcinoma
31	Pneumonia, basilar, bilateral	Emphysema; cor pulmonale; duodenal ulcer	*D. pneumoniae*	0.62	Nov. 24, 1959	Afebrile	Improved	Breathing improved and cough less productive by Dec. 2, 1959
35	Pneumonia, LLL	Emphysema; myocardial infarction, old	*D. pneumoniae*	0.62	Nov. 20, 1959	3 days		Died on Nov. 23, 1959, autopsy confirmed pneumonia, LUL
51	Pneumonia, LLL		*D. pneumoniae*	0.07	March 2, 1960	4 days	Cured	
53	Pneumonia, RML	Emphysema; bronchiectasis	*D. pneumoniae*	0.15	Feb. 6, 1960	4 days	Improved	Decrease in amount of usual sputum, died March 15, 1960, of acute suppurative bronchopneumonia
54	Pneumonia, RLL		*D. pneumoniae*	0.07	Feb. 9, 1960	2 days	Cured	
56	Pneumonia, lower lobes	Pancytopenia, etiology unknown	*D. pneumoniae*	0.07	Feb. 10, 1960	1 day	Cured	
29	Pneumonia, RLL	Calcification of gall bladder			Sept. 11, 1959	1 day	Cured	Smear suggested pneumococcus; coliform from sputum after treatment MIC > 10
44	Pneumonia LLL	Multiple sclerosis			Nov. 17, 1959	24 hours	Cured	Culture lost; smear suggested pneumococcus
32	Pneumonia, RUL	Subleukemic leukemia			Oct. 30, 1959	1 day		Smear suggested pneumococcus; on Nov. 11, *S. aureus* pneumonia developed and he died on Nov. 24

*Minimal inhibitory concentration of DMCT (μg per milliliter of serum).

Table I. *Cont'd*

Case no.	*Diagnosis*	*Other diagnoses*	*Organism*	MIC*	*Date DMCT started*	*Fever to normal*	*Result*	*Comment*
5	Pneumonia, five lobes	Alcoholism	*K. pneumoniae*	2.5	Nov. 11, 1959	36 hours		Died Nov. 12, 1959; also received penicillin, chloramphenicol, streptomycin
40	Pneumonia, LLL	Hodgkin's disease	Normal flora		Oct. 31, 1959	24 hours	Improved	Died 10 days after therapy concluded of multiple pulmonary emboli
22	Bronchopneumonia, bilateral	Bronchiectasis	Normal flora		Oct. 3, 1959	7 days	Improved	
42	Pneumonia, lower lobes	Emphysema; latent syphilis; arteriosclerotic heart disease	Normal flora		Dec. 24, 1959	1 day	Cured	*S. aureus*, coagulase-positive, isolated from sputum during therapy MIC > 10
28	Pneumonia, LLL		Normal flora		Nov. 24, 1959	No fever	Cured	Penicillin, single injection Nov. 24
17	Pneumonia, RUL	Emphysema; epilepsy	Normal flora		Dec. 20, 1959	2 days	Cured	
16	Pneumonia, LLL	Chronic lymphocytic leukemia	Normal flora		Jan. 5, 1960	1 day	Cured	
47	Pneumonia, LLL	Emphysema	Normal flora		Jan. 11, 1960	No fever	Improved	Sputum became clearer and less copious; Pseudomonas in sputum after therapy
52	Pneumonia, LLL	Emphysema	Normal flora		Feb. 3, 1960	9 days	Improved	Less cough, sputum, wheezing
50	Pneumonia, RUL		Normal flora		Feb. 1, 1960	7 days	Cured	Lobar pneumonia with profuse bloody, purulent sputum
21	Pneumonia, RLL	Asthma; cirrhosis	Normal flora		Nov. 9, 1959	4 days	Improved	Yeast in sputum after therapy
10	Pneumonia, RLL	Adenocarcinoma lung; alcoholism	Normal flora		Dec. 1, 1959	4 days	Improved	Subsequently died from carcinoma, lung
49	Bronchopneumonia	Emphysema; carcinoma, prostate	Normal flora		Jan. 26, 1960	3 days	Improved	

Table I. *Cont'd*

Case no.	*Diagnosis*	*Other diagnoses*	*Organism*	*MIC**	*Date DMCT started*	*Fever to normal*	*Result*	*Comment*
46	Pneumonia, LLL	Diabetes mellitus; gout; cerebral arterio-sclerosis	Normal flora		Sept. 25, 1959	6 days	Improved	
59	Pneumonia, RUL	Asthma	Normal flora		Nov. 11, 1959	Afebrile	Improved	Slow resolution by x-ray, *A. aerogenes* in sputum after treatment MIC > 10
48	Pneumonia ?	Emphysema; arterio-sclerotic heart disease; pneumo-thorax	Normal flora		Jan. 21, 1960	24 hours	Improved at first	Developed *S. aureus* empyema and died Feb. 19, 1960
57	Pneumonia, RUL	Adenocar-cinoma, right lung	Pseudo-monas		Feb. 13, 1960	7 days	Unchanged	Low grade fever re-curred after drug stopped
12	Pneumonitis, RUL	Pulmonary tubercu-losis, old; fracture, hip	Pseudo-monas		Sept. 30, 1959	24 hours	Improved	Also received peni-cillin and strep-tomycin first 12 hours
14	Pneumonia, RUL	Broncho-genic car-cinoma, RUL	*A. aerogenes*	0.625	Aug. 18, 1959	Afebrile	Improved	Cough and sputum production less-ened
58	Pneumonia, RLL	Emphysema; arterio-sclerotic heart disease	*E. coli*		Feb. 19, 1960	48 hours	Cured	
38	Pneumonia, LLL	Mesothe-lioma, right lung	Proteus		Feb. 19, 1960			Drug stopped be-cause of persistent nausea with vom-iting present be-fore drug started
7	Pneumonia, RLL	Asthma	*S. aureus*, coagulase-positive		Jan. 20, 1960	4 days		Died on tenth day; lungs sterile at autopsy
1	Broncho-pneumonia	Emphysema; carcinoma, bladder	*S. aureus*, coagulase-positive	>10	Sept. 29, 1959	13 days	Improved	
43	Pneumonia, right lung	Adenocar-cinoma, right lung			Dec. 20, 1959	4 days	Improved	No sputum culture obtained; blood sterile
45	Bronchiec-tasis	Carcinoma, esophagus	Proteus	0.625	Sept. 22, 1959			Died 12 hours after therapy started

Material and methods

Between July, 1959, and February, 1960, 59 patients with pulmonary, urinary tract, or skin infections received DMCT* orally. Upon suspicion of infection, initial cultures, blood count, urinalysis, and various liver and renal function tests were performed. Patients were then placed on one of three general oral treatment dosages: 150 mg. every 6 hours, 300 mg. every 8 hours, or 300 mg. every 12 hours. Various "loading" doses were used, and most patients received the drug for a total of 10 days. No specific effort was made to give the medication before or after meals, with or without milk. After treatment, the laboratory tests were repeated. No double blind techniques were used. Most of the patients were not told that they were receiving a new antibiotic. Tube dilution tests for bacterial sensitivity to DMCT were performed.

Results

Pulmonary infections. Thirty-seven patients with pneumonitis (36 men and 1 woman) ranging from 38 to 86 years of age were studied. Organisms recovered from the sputum were diplococcus pneumoniae (9 cases), Friedländer's bacillus (1), other Gram-negative bacilli (5), *Staphylococcus aureus* (3), *Candida albicans* (1), and "normal flora" including alpha streptococci, *Neisseria catarrhalis*, diphtheroids, and *Staphylococcus albus* (18) (Table I).

Pneumococcal pneumonia. Nine sputum-positive patients were treated. One also had a positive blood culture. All were men of ages ranging from 40 to 67 years. They were treated from 3 to 10 days. Eight were cured of the infection, one died. The one death (case 35) was in a 64-year-old white man who had left upper lobe pneumonia, obstructive emphysema, and severe hyponatremia (serum sodium 115 mEq.). He died suddenly on the third day of therapy, and autopsy confirmed the presence of a left upper lobe pneumonia, emphysema, and pulmonary arteriosclerosis with cor pulmonale. The response in 4 of the other 8 patients was slow, and the temperature did not fall to normal until at least the fourth day after the beginning of therapy. It is of interest that viable pneumococci were isolated from the sputum of 1 patient (case 18) who had 300 mg. of DMCT every 6 hours for 6 days. This patient's temperature did not return to normal until the seventh day.

Three additional patients with pneumonia, 1 woman, and 2 men, ages 45 to 77 years, were suspected of having pneumococcal pneumonia both clinically and by direct smear of sputum, but the organism was not isolated by culture. Two of these were clinically cured. The third patient (case 32), a 64-year-old white man with subleukemic leukemia and right upper lobe consolidation, initially had a good response. However, on the eleventh day of therapy, he again became febrile and developed patchy consolidation of the left upper lobe. *S. aureus*, coagulase-positive, was grown from his sputum and, despite large doses of several antibiotics, he died on the twenty-fourth day.

Friedländer's pneumonia. A 38-year-old white male alcoholic with Friedländer's pneumonia (case 5) died 36 hours after admission. In addition to DMCT, he received penicillin, chloramphenicol, streptomycin, and parenteral 6-methyl-delta-1-hydrocortisone.*

Other Gram-negative organisms. In all 5 patients (cases 12, 14, 38, 57, and 58) where other Gram-negative organisms were isolated from sputum, there was underlying pulmonary disease (3 with lung cancer, 1 with old pulmonary tuberculosis, and 1 with obstructive emphysema). All were men aged 65 to 78 years. Three of these were symptomatically improved as judged by the amount and character of the sputum and fever, 1 was unchanged, and 1 took the medication less than 24 hours because of

*Declomycin.

*Solu-Medrol.

Table II. *Patients with urinary tract infections treated with demethylchlortetracycline*

Case no.	*Diagnosis*	*Other diagnoses*	*Organism*	MIC*	*Date DMCT started*	*Fever to normal*	*Result*	*Comment*
6	Pyelonephritis, acute	Bronchitis, post-TUR	Proteus	>10	Aug. 10, 1959		No change	Proteus in urine at end of therapy
8	Pyelonephritis, acute	Pancytopenia, etiology unknown	Achromobacteriaceae		Oct. 29, 1959	48 hours	Cured	
37	Pyelonephritis, acute	Arteriosclerotic heart disease; aortic aneurysm	*A. aerogenes*	2.5	Aug. 12, 1959	Gone when drug started	Improved	Urine was made sterile
3	Pyelonephritis, chronic	Diabetes mellitus	*A. aerogenes*	0.31	Oct. 15, 1959	None	Improved	Urine was made sterile
27	Pyelonephritis, chronic	Cirrhosis; duodenal ulcer	*E. coli*	0.62	Oct. 7, 1959	None	Improved	Urine was made sterile
1	Cystitis	Emphysema; carcinoma, bladder	Proteus	>10	Sept. 29, 1959	13 days	No change	On catheter drainage; urine grew Proteus after therapy
13	Cystitis	Tabes; cardiovascular anomaly	Proteus	>10	Oct. 14, 1959	None	No change	Urine grew Pseudomonas after therapy; died with terminal pneumonia Oct. 26, 1959
36	Cystitis	Chronic lymphocytic leukemia	*E. coli*		Sept. 8, 1959	None	No change	Urine grew *A. aerogenes* after therapy
11	Cystitis	Prostatitis; myocardial infarction	*E. coli* (pretreatment); Proteus (post treatment)	1.25 >10	Sept. 2, 1959	None	No change	Urine grew Proteus after therapy
15	Cystitis	Arteriosclerotic heart disease; diabetes mellitus; benign prostatic hypertrophy	Paracolon		Sept. 3, 1959	None	Improved	Urine was made sterile
23	Cystitis	Cirrhosis; psoriasis			Oct. 23, 1959	4 days	Improved	Urine was sterile
4	Cystitis	Cirrhosis			Oct. 23, 1959	24 hours	Improved	Urine was sterile

*Minimal inhibitory concentration of DMCT (μg per milliliter of serum).

persistent vomiting (present before the drug was started).

S. aureus. In 2 patients (cases 1 and 7), both men, aged 67 and 74 years, *S. aureus*, coagulase-positive, was initially isolated from the sputum. In each, underlying pulmonary disease was also present (asthma and obstruction emphysema). One of these patients, with severe asthma, died at the end of 10 days of therapy, and at autopsy, lung cultures were sterile. The other patient was symptomatically improved, but at the conclusion of therapy *S. aureus* was still present in the sputum.

Normal flora. In 15 patients, all men, aged 40 to 86 years, only "normal flora" was isolated from the sputum. Eleven of these had leukocytosis with a shift to the left, purulent sputum, fever, and x-ray evidence of pneumonitis. These cases were thought to be bacterial in nature. Six patients were cured after therapy, and 5 were improved (2 had emphysema, 1 asthma, 1 Hodgkin's disease, and 1 adenocarcinoma of the lung). Of the other 4 patients with fever, dyspnea, râles, and increased white blood cell count, no definite pulmonary infiltrates were seen in 2. One patient (case 48) developed an empyema (*S. aureus*, coagulase-positive) 13 days after cessation of a 13 day course of DMCT. Three days later he died.

Others. Two additional male patients received the drug for pulmonary infection. One (case 45) with far advanced bronchiectasis died 12 hours after DMCT therapy was started, and 1 (case 43) with adenocarcinoma of the lung did not have sputum cultures made.

Urinary tract infections. Twelve patients with urinary tract infection were studied (Table II). All were men; ages varied from 40 to 74 years. Acute pyelonephritis was present in 3 (cases 6, 8, and 37), the chronic form in 2 (cases 3 and 27). In four of the 5 cases of pyelonephritis, the urine was sterile at the conclusion of therapy. No long term follow-up cultures were obtained. Cystitis was diagnosed in 5 patients (in cases 1, 13, and 36 after catheterization, in case 11 with prostatitis, and in case 15 with benign prostatic hypertrophy and hematuria). In only *1* of the 5 patients (case 15) was the urine free from bacteria at the end of treatment. The four persistent cases of bacteruria were associated with organisms resistant to DMCT in vitro. Two additional patients (cases 4 and 23), thought clinically to have urinary tract infections, had sterile urine both before and after therapy.

Cellulitis. Eleven patients received DMCT for what was considered to be cellulitis, without significant effect or evaluable results (Table III).

Sensitivity studies on organisms isolated

The tube dilution tests for bacterial sensitivity to DMCT indicated that the organisms tested were either very sensitive or completely resistant. The latter included certain strains of *S. aureus*, coagulase-positive, and *Proteus* and *Aerobacter aerogenes.* Intermediate levels of susceptibility were rarely encountered. No attempt was made to compare in vitro susceptibility to DMCT and other tetracyclines.

Other effects

Laboratory tests. Several different "paired laboratory tests," taken at the start and the conclusion of therapy with DMCT, were analyzed. There was no observed leukopenia, fall in hemoglobin, proteinuria, pyuria, or hematuria which could be attributed to drug toxicity. Liver function studies included sulfobromophthalein sodium excretion, cephalin flocculation, and alkaline phosphatase determinations. Of 43 paired determinations of sulfobromophthalein sodium excretion (normal: up to 12 per cent in 45 minutes), 2 patients (cases 12 and 46) showed unexplained rises of from 29 to 54 per cent and 12 to 52 per cent in 45 minutes. Of 44 paired cephalin flocculation tests (normal: 0 to 2+ in 48 hours), 3 patients (cases 6, 20, and 39) showed unexplained rises of 0 to 3+ in 48 hours and 1 (case 22) from 1+ to 3+. Of 45 paired serum alkaline phosphatase tests

(normal: 0.5 to 3.0 Bessy-Lowry units), 1 (case 44) showed an unexplained rise from 2.7 to 3.8 Bessy-Lowry units. Renal function tests included urinalysis and determination of blood urea and serum creatinine levels. Of the 46 paired blood urea determinations (normal: 10 to 20 mg. per 100 ml.), 7 showed unexplained rises of more than 7 mg. per 100 ml., 4 of these (cases 18, 44, 47, and 57) from normal to abnormal and 3 (cases 22, 46, and 53) additional rises of already abnormally elevated levels. One patient (case 46) showed a rise from 35 to 74 mg. per 100 ml. Of the 46 paired serum creatinine determinations (normal: 0.5 to 1.5 mg. per 100 ml.), 5 patients (cases 12, 18, 25, 39, and 46) showed unexplained elevations. Four of these rose up to 1.8 mg. per 100 ml. and 1 (case 46) rose from 1.6 to 2.1 mg. per 100 ml.

None of these abnormalities in laboratory results were followed up with additional tests. The most severe abnormalities in sulfobromophthalein sodium excretion, blood urea, and serum creatinine levels occurred in 2 patients (cases 12 and 46).

Gastrointestinal effects. Gastrointestinal disturbances were noted in several cases. After eliminating the five deaths occurring during the therapy and the single case with nausea at the start of therapy, 53 cases remained. Fourteen, or 26.5 per cent, of the patients had gastrointestinal complaints including "fullness, anorexia nausea, vomiting, and diarrhea." Nine of these 14 had mainly subjective complaints, whereas 2 developed vomiting and 3 diarrhea. In no case was it necessary to discontinue the medication because of this. Six of 10 patients who received 200 mg. every 6 hours for 3 days, followed by 300 mg. every 8 hours for 7 days, complained of anorexia and nausea. Thus, it appeared that the frequency of gastrointestinal disturbances was a function of the daily dose of DMCT.

Discussion

The tetracyclines have wide popular appeal for a variety of infections, particularly because of the ease of oral administration and their apparent effectiveness. The recent addition to this group, demethylchlortetracycline, has an antibacterial spectrum similar to the other tetracyclines.[4, 5] The higher and more prolonged blood levels observed with equivalent oral and intravenous doses are felt to be a unique advantage.[2-5]

Perhaps the most significant aspect of the present study is the illustration of the very great difficulty faced by the physician who attempts to evaluate a new antimicrobial drug which resembles other good drugs. A majority of patients with possible infection seen in either private practice or in a large general hospital do not have simple, uncomplicated microbial disease in which the outcome of antimicrobial therapy could be readily evaluated. On the contrary, many patients with infection suffer from underlying illnesses which predispose them to infection or modify the infectious process through alteration of anatomic or physiologic host conditions. In this complex situation, the suppression of microbial growth—the sole effect of an antibiotic—contributes only to a variable and unpredictable extent to the relief of symptoms and signs of infection. Thus, the difficulties of assessing the merits of an antimicrobial drug in the *prevalent* patients are great. In spite of these recognized problems of evaluation and in spite of the limited number of patients observed, we have formed some impressions regarding the activity of oral DMCT.

By laboratory test, microorganisms recovered from our patients clearly fell into two groups: Some (particularly pneumococci, streptococci, and occasionally coliform organisms and staphylococci) were inhibited by very small amounts of DMCT. Others (certain strains of staphylococci and coliform bacteria) grew freely in 10 μg DMCT per milliliter. Only very few strains were intermediate in susceptibility. In general, patients tended to respond when the organisms were highly susceptible and failed to improve if the organisms were completely resistant.

Table III. *Patients with skin infections treated with demethylchlortetracycline*

Case no.	*Diagnosis*	*Other diagnoses*	*Organism*	*MIC**	*Date DMCT started*	*Fever to normal*	*Result*	*Comment*
20	Cellulitis, feet	Epidermophytosis	S. *aureus*, coagulase-positive	0.15	Oct. 12, 1959	None	Cured	
24	Cellulitis, arm		S. *aureus*, coagulase-positive	0.312	Oct. 5, 1959	None	Cured	
19	Cellulitis, ears	Porphyria cutanea tarda	Beta-hemolytic streptococci; S. *aureus*, coagulase-positive		Oct. 5, 1959	None	Cured	
55	Cellulitis, right leg	Alcoholism; delirium tremens	Beta-hemolytic streptococci; S. *aureus*, coagulase-positive	0.62	Feb. 8, 1959	3 days	Worse	Cellulitis spread during therapy; penicillin and chloramphenicol cured infection in 5 days
34	Cellulitis, after burn		Beta-hemolytic streptococci		Nov. 9, 1959	None	Improved	Also received penicillin
41	Cellulitis, right leg	Diabetes mellitus			Sept. 22, 1959	None	Improved	
25	Abscess, buttock	Hookworm infestation	S. *aureus*, coagulase-positive	0.15	Dec. 3, 1959	3 days	Cured	Fever disappeared when abscess incised and drained
39	Abscess, leg	Thrombophlebitis	S. *aureus*, coagulase-positive	0.312	July 31, 1959		Cured	Also received penicillin and abscess was incised and drained
30	Cellulitis	Splenectomy, 1952	Alpha streptococci	0.31	Oct. 10, 1959		Cured	Incised and drained also
33	Fever of unknown origin	Delirium tremens			Oct. 25, 1959	Persistent fever	No change	Prolonged fever finally attributed to delirium tremens
9	Fever of unknown origin	Diabetes mellitus; myocardial infarction			Dec. 6, 1959	Persistent fever	No change	Probable viral illness

*Minimal inhibitory concentration of DMCT (μg per milliliter of serum).

Where the organism was sensitive in vitro, the minimal inhibitory concentration was in the range reported to be attainable by average doses of DMCT.[2-5]

Pneumococcal pneumonia showed a favorable response, though probably somewhat slower than that observed with penicillin. In 1 case, viable pneumococci were isolated from the sputum on the sixth day of therapy. "Mixed" pulmonary infections in which no pathogen was isolated, occurring usually where primary lung disease was also present, showed uniform improvement. Although 1 patient with Friedländer's pneumonia died, he was a chronic alcoholic and the disease was advanced when first observed. He also failed to respond to the later addition of chloramphenicol and streptomycin, and it is doubted that any form of therapy could have prevented his death. Other Gram-negative infections, either of the lung or urinary tract, showed a favorable response to the drug when there was evidence of in vitro sensitivity of the infecting organism.

Staphylococcal and streptococcal skin infections were favorably affected except for 1 case. Incision and drainage of an abscess was felt to be more important than administration of the antibiotic. In 2 patients, staphylococci isolated from the sputum were resistant to this drug and in 1 were not eradicated from the sputum with this therapy. As has been the experience with other tetracyclines, a staphylococcal superinfection may occur during use of DMCT (2 cases in this series), and throat or sputum cultures after treatment may show yeast (2 cases) or Gram-negative organisms (6 cases).

There is a suggestion of hepatic and renal toxic effect by the drug. A few abnormalities, as listed, in sulfobromophthalein sodium excretion, cephalin cholesterol flocculation, and urea and creatinine levels were observed. Lack of follow-up studies makes uncertain the true significance of these abnormalities. Large intravenous doses of tetracyclines may cause moderate fatty infiltration of the liver, clinical jaundice, and impairment of hepatic function.[6] Also, transient azotemia has been observed, chiefly in patients with impaired kidney function, and appears to be related to the metabolic effects of the tetracyclines.[6] After oral administration of DMCT, rises in nonprotein nitrogen have been observed by Lichter and associates,[5] though they felt that "in none of the patients was evidence of hemopoietic, hepatic, or renal toxicity apparent."

Gastrointestinal symptoms, though occurring in 26.4 per cent of the total series (deaths not included), were minor, and in no case did the drug have to be discontinued because of them. With doses of DMCT in excess of 600 mg. daily, gastrointestinal effects were quite prominent.

Conclusions

A clinical evaluation of the new tetracycline, demethylchlortetracycline, is presented. This drug appears to be comparable to other tetracyclines in bacterial in vitro sensitivity patterns. Patients who had infections of the lungs, urinary tract, or skin caused by a sensitive organism in the main responded favorably. Two superinfections with staphylococci occurred. A high incidence of minor gastrointestinal disturbances was noted. A suggestion of renal and hepatic toxicity requires further investigation.

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Prof. V. H. Blackman (1956) writes:

"At a meeting of the Evolution Committee of the Royal Society, Weldon had read a paper on the sizes of the carapaces of a certain population of crabs. Bateson, who considered the results of no biological importance, when asked by the Chairman of the Committee to comment upon Weldon's contribution did so in a single devastating sentence: 'though all science might be measurement, all measurement is not necessarily science.'"

Professor Blackman adds:

"I may say I have occasionally found it useful to bring this dictum before research students, who are sometimes inclined to believe that so long as they are measuring something they must be advancing science."

FROM "THE STRATEGY AND TACTICS OF EXPERIMENTATION"
BY C. W. HUME, LANCET, VOL. 2, P. 1049, 1957.

Prolonged coma caused by glutethimide

The clinical course of a patient who ingested a large amount of glutethimide is described. The patient remained in a coma for 6 days but ultimately made a complete recovery. In the management of the patient, meticulous attention to the airway, pulmonary ventilation, and maintenance of blood pressure is emphasized. The usefulness of the Morsch respirator is described. Reports of similar patients in the literature are reviewed.

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The case report presented here demonstrates virtually all of the clinical aspects of overdosage with glutethimide.* Glutethimide is a relatively new hypnotic compound generally considered to be safe. The experience cited here and a summary of reported cases should make it clear that it is a drug which should be administered with care. Knowledge of the therapeutic measures employed in the care of the prolonged coma exhibited by this patient may be useful to others with similar problems.

Case report

A 57-year-old white woman was admitted to Saint Vincent's Hospital for the first time on March 28, 1960, at 7:45 P.M. She was comatose. Her sister revealed that the patient had been severely depressed, had been under psychiatric care, and 2 days prior to admission had filled a prescription for fifty tablets of glutethimide (500 mg. each). The patient was last known to be conscious at 12:00 noon on the day of admission. She was found in a coma with the empty bottle of glutethimide beside her. On admission, physical examination revealed a well-developed, slightly obese, white woman who was comatose and unresponsive. Blood pressure was 90/70 mm. Hg. The pulse rate was 60 per minute, and the temperature was 98° F. Respirations were 16 per minute and shallow. Pupils were dilated and did not react to light. The fundi were normal. Neurologic examination revealed the deep tendon reflexes were absent. No abnormal reflexes were noted.

Intravenous glucose (5 per cent in water) was started and vital signs were regularly recorded. Thirteen hours after admission, blood pressure was 60/0 mm. Hg, pulse rate was 100 per minute, and respirations were 24 per minute and very shallow. Slight cyanosis was noted; deep tracheal suctioning was done, and the cyanosis temporarily cleared. An infusion of 1,000 ml. of 5 per cent glucose in water containing 4 ml. of a 1 per cent solution of phenylephrine was begun. Blood pressure returned to normal levels promptly.

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*Doriden.

Tracheostomy was performed 18 hours after admission, because of copious bronchial secretions. Respirations later became inadequate and cyanosis was noted; the Bennet respirator was attached to the tracheal airway 21 hours after admission. However, because of the technical difficulty in managing the tracheostomy in this obese, short-necked woman, the patient-respirator circuit could not be maintained closed. The Morsch respirator,[1] a positive-negative apparatus depending solely on its cylinder piston mechanism for inflating and deflating the lungs, was substituted and set to cycle 20 respirations per minute with a tidal volume of 2,500 ml. of room air.

Pressor agents were required to maintain blood pressure during the first few days in the hospital. At the beginning of the second day, the deep tendon reflexes were present and symmetric. No pathologic reflexes appeared, and there was no other change on physical examination. Toward the end of the second day, a lumbar puncture was performed. The initial pressure was 500 mm. of water. The fluid was clear, it contained no cells, and the protein content was 40 mg. per 100 ml. During the third day in the hospital, it was noted that the patient responded to very painful stimuli by withdrawal of all limbs except the right arm. The pupils were in mid-dilation and responded sluggishly to light.

On the fourth day, bilateral Babinski reflexes were noted without any other changes in status. Lyophilized urea, 180 Gm. in 420 ml. of 10 per cent invert sugar, was administered over an 8 hour period. A marked diuresis ensued, and fluid intake was then severely restricted. After 8 hours, it was noted that the patient turned her head in the direction of verbal stimuli. There was no other change in physical or neurologic examination at this time, and within an hour the patient was again responsive only to very intense pain, from which she withdrew all extremities except the right arm. A third lumbar puncture was then performed. The initial pressure was 190 mm. of clear fluid without cells. During the fifth and sixth days, the patient at times responded to questions and asked for sips of water, yet at other times would respond only to painful stimuli. The patient

Table I. *Hospital course*

Determination	*Hours*						
	4-28	*28-52*	*52-76*	*76-100*	*100-124*	*172-196*	*196-220*
Intake (ml. per 24 hr.)	2,975	2,825	1,800	2,325	2,025	1,600	2,500
Output (ml. per 24 hr.)	2,495	4,235	2,060	3,980	3,820	1,000	1,400
Blood urea nitrogen (mg. per 100 ml.)		22			81	24	
Sodium (mEq. per L.)		146	136	146	140	145	140
Potassium (mEq. per L.)		5.1	5.4	5.1	2.8	3.7	4.0
Chloride (mEq. per L.)		108	103	109	113	104	104
Carbon dioxide (mEq. per L.)		23	19	17	17	24	32
pH serum	7.37	7.33		7.34	7.43		7.38
Blood creatinine (mg. per 100 ml.)	1.0						
White blood cells	9,100			13,400			11,700
Urine	Normal			Normal			Normal

Table II. *Summary of pertinent data reported in cases of glutethimide poisoning*

Source	*Sex*	*Age*	*Grams of glutethimide ingested*	*Condition of pupils*	*Blood pressure*	*Respirations*
Blakey[2]	F	39	10	Mydriatic	Below normal	Apneic
	M		20			
Bordleau[3]	M	29	6			
	F	31	6			Shallow
	F	23	10	Mydriatic		
Burnstein[4]	M	38	5	Normal	Normal	Normal
Chandler[5]	F	19		Mydriatic	Normal	Rapid, shallow
	F	34		Mydriatic	Below normal	Slow, shallow
Gerster[6]	M	20	15	Mydriatic		Apneic
McBay[9]	F		5	Mydriatic	Below normal	Rapid, shallow
	M		15			
	M		12			
		33	50			
Schreiner[3]	F	31	7.5	Mydriatic	Normal	Rapid, shallow
	F	36	10	Mydriatic	Below normal	Slow, shallow
	F	24	10	Mydriatic	Below normal	Depressed
	F	44	6	Mydriatic	Below normal	Shallow
	M	47	12	Mydriatic	Below normal	Rapid
	F	39	18	Mydriatic	Below normal	Rapid, shallow

was able to take a blender diet from a bulb syringe by the sixth day. She was able to stand and walk with some support on the ninth day. At this time, neurologic examination results were entirely within normal limits. In the ensuing days lethargy cleared, and the patient's friends judged her mental acuity and behavior to be comparable with that observed prior to coma.

Table I shows the laboratory data and other pertinent observations made during hospital course.

Comment

This patient presented many of the features reported by others as occurring in the course of glutethimide overdose (see Table II). Thus, hypotension, insufficiency of either depth or rate of respiratory movements, vexatious bronchial secretions, prolonged coma and relapse into a comatose state after partial recovery, the presence of deep tendon reflexes despite nonreactivity to pain, mydriases, signs of focal cerebral injury which clear upon recovery from coma, facial grimacing, and unilateral twitching of an extremity or of the facial muscles are all relatively characteristic of severe glutethimide intoxication.

The pharmacologic nature of this drug is incompletely known.[2, 7, 8, 10] Glutethimide is a white crystalline powder insoluble in water. When ingested, it is slowly and incompletely absorbed and probably bound to the tissues. It is slowly excreted via the kidneys after detoxification in the liver. It is not known to be hepatotoxic. In animal studies, it has been shown to depress both central and autonomic nervous system functions with varied effects. Regularly, the animals arouse from sleep more easily than with barbiturates. Because of considerations of molecular size, glutethimide is able to pass through semipermeable membranes.

The therapy of coma resulting from glutethimide must aim at the support of blood pressure, maintenance of pulmonary ventilation, and adequate cleansing of the bronchial tree. Infections, should they arise, must be vigorously treated. If urinary out-

Bronchial secretions	*Reflexes*	*Body temperature*	*Result of treatment*
Increased	Normal		Improved after 2 to 3 days
			Death
Increased			Improved
Increased	Normal		Death after 48 hours
Increased	Normal		Improved after 2 to 3 days
Normal	Normal	Normal	Improved after 24 hours
Increased	Normal	Elevated	Improved after renal dialysis
Increased	Depressed	Normal	Improved after renal dialysis
	Absent	Elevated	
	Absent		Improved after 36 hours
			Death
			Death
			Death
			Death
Increased	Depressed	Elevated	Alert after 48 hours
	Depressed		Improved after dialysis
Normal	Depressed	Normal	Death after 69 hours
Increased	Depressed	Normal	Recovered after 48 hours
Increased	Normal	Normal	Recovered after 70 hours
Increased	Absent	Normal	Dialyzed after 90 hours

put cannot be maintained, renal hemodialysis should be considered. Gastric lavage should be done, since it has been demonstrated that delayed absorption from the intestines produces significant blood levels 24 to 48 hours after coma develops.[5, 7] Schreiner[10] reports that apnea or pulmonary aspiration may follow minimal pharyngeal stimulation in these patients. Hence, gastric lavage should be avoided until tracheotomy is performed or an endotracheal tube is in place. All autopsies in patients with glutethimide overdosage have revealed significant cerebral edema,[2, 7, 9] and the majority of patients that survive have shown transient neurologic signs. Thus, cerebral edema should be considered a potential complication, and specific therapy may be necessary.

Of greatest aid in the case presented was the Morsch respirator.[1] It provided ideal pulmonary ventilation, while it also permitted adequate tracheal suction, adjustment of the posture of the patient, and full access to the patient for examination, special therapy, and nursing care. Another aspect of interest was the use of lyophilized urea prompted by the appearance of focal signs of neurologic injury. Though spinal fluid pressures were not observed frequently, there was some suggestion of significant lowering of the spinal fluid pressure and some lightening of coma shortly after the administration of the drug. Gastric lavage was not done. However, when one considers the great duration of coma exhibited by the patient, the large amount of drug ingested, and its slow absorption from the intestines, this may have been a mistake. Schreiner[10] demonstrated the ability of bemegride* to counteract the effects of glutethimide. Recovery from coma is clearly dependent on excretion of the drug from the body. It would appear from this case that if physiologic support of blood pressure and meticulous attention to ventilation are carried out, hemodialysis need not be undertaken.

*Megimide.

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Anesthetic time/dose curves

II. The limiting factor in the utilization of intravenous anesthetics during surgical operations

The construction of mean time/dose curves of intravenous anesthetic drugs during surgical operations under standardized conditions is a simple and sensitive way to determine the equipotent doses of anesthetic agents. Such curves were determined for meperidine and oxymorphone, and the results were compared with the corresponding data previously established for thiopental.

In spite of differences in the anesthetic potency of these agents by a factor approaching 500, the mathematical characteristics of the time/dose curves correspond so closely as to suggest that under the conditions of this study, a common factor limits the drug requirements.

Arguments are presented in favor of the hypothesis that the common factor might be the effective blood flow through the liver or the permeability of the hepatic cell membrane.

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In a previous study,[6] we determined the thiopental requirements of unselected surgical patients during operations lasting up to 640 minutes, and we presented a mathematical analysis of the resulting time/dose curve. Beyond certain conclusions regarding the fate of thiopental, we hoped that the time/dose curve would find application as a tool in clinical investigation. This report is concerned with the results obtained with meperidine and oxymorphone,* the problems that were raised, and their tentative explanations.

Material and methods

The investigations were carried out on otherwise unselected surgical patients whose anesthesia was expected to last longer than 3 hours, who represented as a group, therefore, worse than average surgical risks. They were premedicated with 100 mg. of a barbiturate orally on the morning of surgical operation, at least 100 minutes before the start of anesthesia. The

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*Numorphan.

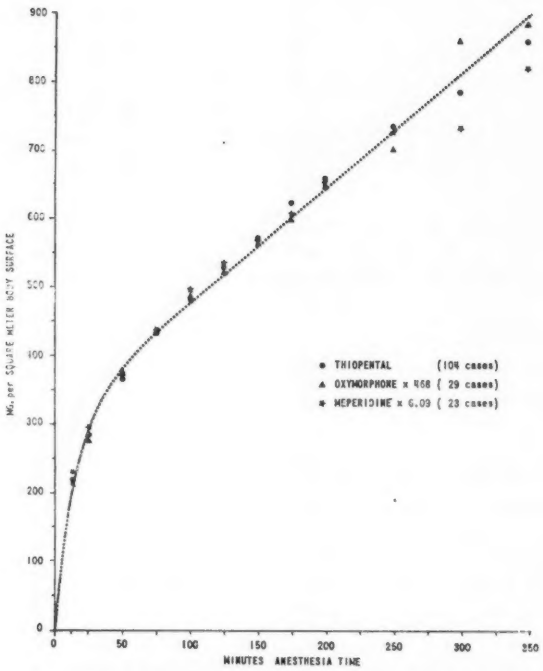

Fig. 1. The mean time/dose curve of thiopental, meperidine, and oxymorphone. A correction factor has been applied to the meperidine and oxymorphone data to compensate for differences in relative potency (see Table II) and to permit the presentation of the three drugs on a common graph.

barbiturate was pentobarbital, secobarbital, or phenobarbital, depending upon the time of the day for which the operation was scheduled. At 75 minutes before the start of anesthesia, the patients received 50 mg. promethazine and 0.4 mg. scopolamine intramuscularly. Timing of the injection was kept as constant as feasible in a busy hospital, but the dosage was scaled up or down when extremes of age, size, or physical condition were encountered. Upon arrival of the patient in the operating room, the effect of the premedication was rated on an arbitrary scale from 1 (inadequate) to 5 (overdose) and the result recorded on the patient's chart. Meperidine was employed in a dilution of 5 mg. per milliliter, oxymorphone in the dilution of 75 μg per milliliter. Each drug was used as the only intravenous anesthetic agent for induction as well as for maintenance of anesthesia. They were always administered in conjunction with a standard nitrous oxide–oxygen mixture (5+2 L. per minute for the first 10 minutes and 2+1 L. per minute thereafter) and with a muscle relaxant (0.2 per cent succinylcholine drip) as required. Hyperventilation was a regular feature, either manually or with a respirator. Although not determined in this series, the terminal expiratory carbon dioxide in comparable cases in another, subsequent study was found to be between 4 and 5 per cent. The depth of anesthesia was adjusted from time to time by small increments of the intravenous agent. From these data and the height and weight of the subjects, the cumulative dose of the drug per square meter body surface was determined and a time/dose curve was plotted for each patient. From the curves, the drug requirement of each patient at set times was established; these interpolated data were averaged and served for the determination of the best-fitting mean time/dose curve with the aid of an electronic digital computer by a modification of the method of least squares. The computer also furnished a measure of the goodness of fit in calculating what percentage of the total variability of the mean values could be accounted for by use of the best-fitting equation.*

Results

Table I gives the details on the patients and the various types of surgical operations performed. Table II compares the previously obtained thiopental data with drug consumption in the meperidine and oxymorphone series. Table III lists the data concerning the best-fitting mean curves. Fig. 1 presents the findings in Tables II and III in graphic form.

Comments

As Table I shows, the patients in the two series are comparable. They are also comparable with the thiopental series, reported

*The author is indebted to Prof. J. St.-Pierre and the staff of the Centre du Statistique de l'Université de Montréal for kindly undertaking this task.

elsewhere[6] as far as the various types of surgical procedures are concerned, but the mean anesthesia time in the thiopental series was 195 minutes, and data for premedication rating and relaxant requirements are not available.

Drug requirements vary greatly within each group as the result of sensitivity differences among the various subjects, the changing intensity of the surgical stimulus in the different operations, and the operating techniques of the surgeons. There were even greater variations among the several drugs because of their different anesthetic potencies (Table II). It was all the more striking that when the mean drug requirements of the three agents were compared at set times, the ratios were remarkably constant (columns T/M and T/O, Table II).

The simplest model for depicting drug requirements necessary to maintain constant blood levels in the presence of simultaneously occurring administration, translocation, and transformation assumes the following mathematical expression:

$$Y = At + B(1 - e^{-kt})$$

where Y (mg. per sq. M.) = the cumulative dose of the drug,
A (mg. per sq. M. per min.) = the rate of apparent transformation,
B (mg. per sq. M.) = the amount of drug contained in its apparent distribution volume,
k ($min.^{-1}$) = the relative rate of translocation,
t (min.) = the duration of anesthesia,
e = the base of natural logarithms.

The adequacy of this equation was previously established for thiopental,[6] and it was anticipated that it would also hold true for other intravenous anesthetics. There was no a priori reason to assume however that k would turn out to be essentially the same for all substances and that the ratio of B to A would also remain the same (Table III), especially since there was a sixfold difference between the potency of thiopental and meperidine and a five hundred–fold difference between thiopental and oxymorphone. Yet, the three time/dose curves are superimposable (Fig. 1). In each of them, the apparent transformation rate (A) represents roughly 0.5 per cent per minute of the drug pool (B).

Columns k and B/A in Table III strongly suggest that the apparent distribution space is the same for all three drugs. Since meperidine and oxymorphone are not known to accumulate in fat, it would follow that under the stated experimental conditions these depots cannot play an important part in the uptake of thiopental either, thus confirming recent work by ourselves and others.[5-7]

The results also point to a practical and relatively precise method for the determination of the relative anesthetic potency of intravenous drugs. The method has the advantages that it requires very little in the way of equipment, that the data are collected under actual clinical conditions, and that the comparison of the relative potency of two drugs is made over the entire range of several hundred minutes. Until now, even partial answers to these questions involved sophisticated statistical methods, elaborate electronic equipment, and the unavoidable narrowing of the sampling material that results when such an interdisciplinary approach becomes necessary.[1]

It is intriguing to pursue the question raised by the time/dose curves: what might be the explanation of the finding that three anesthetic drugs, each of them different in chemical structure and anesthetic potency, are utilized by the organism at almost identical rates?

It appears from our earlier study[6] that of the two variables disappearance and changing requirements, the former is quantitatively much more important, particularly when one deals with average values. The best-fitting curve drawn through the mean time/dose data is substantially a titration curve of the anesthetic's inactivation or more precisely of the change—metabolic or otherwise—that limits the rate with which the anesthet-

Table I. *Effect of premedication and type of surgical operation in the meperidine and oxymorphone series*

Characteristic	*Meperidine*	*Oxymorphone*
Average age	46.9	50.49
Sex (F/M)	13/10	14/15
Average premedication rating	3.28	3.12
Average succinylcholine requirements (μg per sq. M. per min.)	72 (5 patients)	71 (17 patients)
Area of surgical operation		
Upper abdomen (diaphragm, stomach)	5	12
Middle abdomen (biliary tree, bowel)	7	8
Lower abdomen (rectum, bladder, gonads)	5	6
Open chest		2
Other	6	1
	23	29
Average anesthesia time (min.)	264	240

ically active compound is transformed into an anesthetically inactive one.

Since it is our thesis that there is an intrinsic common factor for the three agents, we must exclude the possibility that the curves are influenced by some external common factor, such as the nitrous oxide or the muscle relaxants employed in this study. Although nitrous oxide undoubtedly contributed greatly to the achievement and maintenance of anesthesia beyond the first 10 minutes, it may be considered as a constant factor and will not, therefore, influence the shape of the time/dose curve beyond yielding "found" values at 12.5 minutes slightly higher than calculated from the best-fitting equations (Fig. 1).

In the majority of cases in the thiopental series, the muscle relaxant (*d*-tubocurarine) was administered as a constant thiopental-curare mixture (Baird's solution), while in the remaining cases a succinylcholine drip was employed. It has been our impression, shared by many anesthetists but curiously disregarded in print, that in hyperventilated subjects satisfactory abdominal relaxation can be achieved with relaxant doses that will not abolish movements of the limbs or of the face. In consequence, it was hardly ever a problem to decide whether at a given moment our subjects were too lightly anesthetized or not well enough relaxed. A different sort of proof that muscle relaxants did not limit the anesthetic drug consumption is furnished by the observation that if the succinylcholine requirements of our sub-

Table II. *Average drug requirements (mg. per sq. M. body surface) with thiopental (T), meperidine (M), and oxymorphone (O) at set times (t min.); n_T, n_M, and n_O give the number of cases in the respective series*

t	n_T	*T*	n_M	*M*	n_O	*O*	*T/M*	*T/O*
12.5	104	214	23	37.4	29	0.444	5.72	482
25	104	283	23	48.2	29	0.591	5.87	479
50	100	363	23	60.6	29	0.794	5.99	457
75	87	431	23	71.8	28	0.932	6.00	462
100	83	479	22	81.1	28	1.042	5.91	460
125	68	525	22	87.5	23	1.116	6.00	470
150	62	571	22	93.0	22	1.203	6.14	475
175	53	619	22	99.7	19	1.279	6.21	483
200	47	655	19	106.7	18	1.383	6.05	474
250	26	725	11	119.7	14	1.493	6.06	486
300	10	782	8	120.1	8	1.823	6.51	429
350	5	855	5	134.2	6	1.868	6.37	458
							6.09	468

Table III. *Comparison of the constants for the best-fitting average time/dose curves*

		$Y = At + B(1 - e^{-kt})$				
Anesthetic	*No. of cases*	k *(min.$^{-1}$)*	A *(mg. per sq. M. per min.)*	B *(mg. per sq. M.)*	B/A *(min.)*	*Variability accounted for (%)*
Thiopental	104	0.073	1.75	2.98×10^{2}	170	99.4
Meperidine	23	0.065	2.44×10^{-1}	5.53×10	227	98.5
Oxymorphone	29	0.065	3.56×10^{-3}	6.63×10^{-1}	184	99.4

jects (in micrograms per square meter per minute) are plotted against the anesthesia time, there is no correlation, whereas there is a correlation when anesthetic requirements are plotted in the same manner. Furthermore, a considerable number of cases were encountered in which high anesthetic requirements were coupled with slight relaxant utilization and vice versa. We are therefore satisfied that muscle relaxants will not explain our observations.

It would be tempting to assume that the common limiting factor in the inactivation of the three agents is the inactivating metabolic step itself. Unfortunately, the available biochemical evidence[2, 3] indicates that the metabolic pathways of thiobarbiturates, phenanthrene derivatives, and the piperidine group are quite different, and it would be naive to assume that the anesthetic inactivation occurs in an earlier, thus far undetected, common metabolic step. On the other hand, it appears to be equally naive to assume that of the wide range of possible relative transformation rates, all of the agents thus far examined would cluster around one value by sheer coincidence.

The postulation of a physical limiting factor instead of a chemical one appears more promising because the enzymes implicated in the metabolization of these agents are all to be found in the liver microsomes. Given a comparable series of surgical interventions that require comparable depths of anesthesia and produce comparable degrees of hemodynamic upset, it is probable that the effective transhepatic blood flow and the permeability of the hepatic cell membranes of the intracellular partitions would change to a roughly similar degree with little regard to the chemical nature of the anesthetic employed. Either of these factors might limit the availability of the agents to the inactivating enzyme systems. The existence of a maximum removal capacity for liver cells has recently been demonstrated for humans by Waldstein and co-workers.[8] In terms of their model, the anesthetic agents were administered to our subjects in such a way as to favor the development of an equilibrium well below the maximum transfer rate of the liver. At this level, the clearance of the drugs would be greatly in excess of the effective blood flow through this organ, although it should be noted that several of the individual curves showed the characteristic "splaying" interpreted elsewhere[8] as evidence that equilibrium has been established at a level in excess of the minimum threshold.

Several seemingly isolated data come to mind as one contemplates the possibility that, other things being equal, the intravenous drug requirements are determined by the effective transhepatic blood flow. One thinks first of all of the increased susceptibility of hypovolemic patients to thiopental and also to other intravenous agents. Data relating thiopental requirements to hemoglobin levels[4] could be reinterpreted in this sense because in many cases, low preoperative hemoglobin reflects recent blood loss with secondary hemodilution and contraction of the extracellular

space. We are further reminded of the two pregnant women among our 104 thiopental patients: they scored the two highest transformation rates in the entire series. When these and similar bits of evidence fall in place, a significant step will be made toward the understanding of factors that determine drug requirements during clinical anesthesia and toward the rational choice of agents fitted to particular tasks.

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Clinical pharmacology of the tetracycline antibiotics

Some of the relevant features of the clinical pharmacology of the tetracycline antibiotics are reviewed, and, in particular, the absorption, excretion, and disposition in the body of the four homologues that are currently available for clinical use are compared. The relatively minor differences in the chemical structure of these drugs are accompanied by significant differences in some of their physical properties and by some quantitative differences in their absorption, excretion, and distribution in the body, their activity against different bacteria, and their clinical toxicity.

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In 1948, Duggar reported the isolation of a new antibiotic, chlortetracycline†, and the initial experiences with that antibiotic were presented at the same time by a number of workers.[1] Since that time there has accumulated a voluminous literature, including several monographs,[2-5] on the group of tetracycline antibiotics of which chlortetracycline was the first. In this article it is intended to present primarily a critical analysis of some aspects of the clinical pharmacology of the tetracyclines. For more comprehensive reviews, the reader is referred to the monographs by Lepper,[2] Dowling,[3] Musselman,[4] and Florey[5] and to the recent article by Finland and Garrod.[6]

The proper evaluation of drugs is, at best, a very difficult aspect of clinical investigation, but the complexity of the problem is greatly increased when one is called upon to compare preparations that are closely related chemically and very similar in their activity. Because there is, in general, a close correlation between the in vitro activity of an antibiotic and its clinical effectiveness, the concentrations of antibiotic in body fluids and tissues have been widely used to judge the relative merits of one product over another. Although this approach has merit, it has led to some er-

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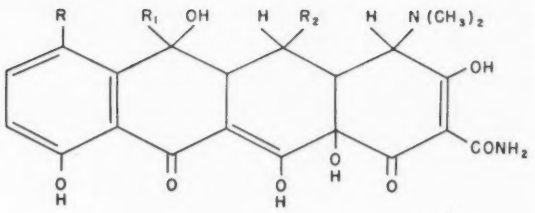
†Aureomycin.

roneous conclusions concerning the clinical significance of some of the supposed advances in chemotherapy that have been reported. The problem of interpreting such reports will also be discussed.

Chemistry

Four tetracycline homologues, chlortetracycline (CTC), oxytetracycline* (OTC), tetracycline (TC), and demethylchlortetracycline† (DMCT), are now available for therapeutic use. Accounts of their isolation and chemical properties are to be found elsewhere.[1, 7-9] In addition, numerous analogues have been prepared, including among others a series of demethyl derivatives[9] and various halogen-substituted compounds,[10] the properties of which are such that their introduction into clinical use has not been warranted. However, two derivatives of tetracycline have also been made available; one is a phosphate complex[11] which was alleged to be absorbed better than the hydrochloride after oral administration; the other and more recent one, N-(pyrrolidinomethyl) tetracycline,[12] is much more soluble, has an isoelectric point of 7.9 in contrast to 4.9 for tetracycline hydrochloride, and is considered to be absorbed better after intramuscular injection.[13] However, the latter, like the hydrochlorides of all of the tetracycline homologues, is given with a local anesthetic to minimize the pain of injection.[14]



	R=	R_1=	R_2=
TETRACYCLINE	H	CH_3	H
CHLORTETRACYCLINE	Cl	CH_3	H
OXYTETRACYCLINE	H	CH_3	OH
DEMETHYLCHLORTETRACYCLINE	Cl	H	H

Fig. 1. Chemical structure of four tetracycline antibiotics.

Interpretation of results of microbiologic assays

The four tetracycline homologues possess virtually identical antimicrobial spectra, but they differ in their relative activity against individual microbial species or even strains.[15] In general, DMCT is the most active, on a weight basis, against a majority of strains of pathogenic bacteria, but the differences are not very great and are probably not of clinical significance except possibly against organisms that are only slightly or moderately sensitive. CTC, however, is the most active against many organisms, notably *Staphylococcus aureus* and *Bacillus cereus,* strains of which are used in standard assays of this group of antibiotics.[16] In the in vitro tests with CTC, particularly in serum and in tissues, this antibiotic is at a considerable disadvantage because of its much greater lability under the conditions of temperature and pH (alkaline) of these tests.[17-19]

Both the broth dilution and agar diffusion methods for assay of these antibiotics in serum tend to minimize the effects of protein binding, the former by the process of dilution and the latter by the use of antibiotic standards diluted in plasma or in an equivalent binding material such as bovine serum albumin.[16] Nevertheless, comparisons of concentrations of the homologues in serum must be made with full consideration of their protein binding since they differ considerably in this respect, as will be seen below. Furthermore, since the optimum pH for antimicrobial activity of these tetracycline antibiotics is 5.5 to 6,[17, 18] the usual disc sensitivity tests, which are carried out on solid media that are more alkaline, would tend to favor the drugs that are more active or more stable on these routinely used media; DMCT is the most stable at the extremes of temperature and pH that could be used.[9]

The importance of the choice of a test

*Terramycin.

†Declomycin.

strain and its relative susceptibility to the antibiotics under study and the even greater importance of using a single standard for comparisons in evaluating the relative merits of one homologue over another are illustrated in Figs. 2 and 3. Fig. 2, *A* and *B*, shows a comparison of the mean concentrations in the serum of 4 healthy young men after a single intravenous injection of 500 mg. of each of the four tetracyclines and is based on one series of assays; the curves in Fig. 2, *A*, show these results expressed in terms of concentrations of the antibiotics administered, and those in Fig. 2, *B*, show the same results calculated in terms of activity of one of these antibiotics, namely, tetracycline.[20] Likewise, in Fig. 3, the curves in both *A* and *B* represent the mean concentrations of antibiotic obtained after single oral doses of 500 mg. of each of the same four antibiotics,[21, 22] Fig. 3, *A*, showing the results expressed as concentrations of the ingested antibiotic and Fig. 3, *B*, the same levels calculated as tetracycline activity. All of these tests were carried out with *B. cereus* as the test organism by a cup-plate method.[16] Against this strain, DMCT was 70 per cent as active as CTC, 3 times as active as TC, and 4 times as active as OTC. Against the strains of *S. aureus* ATCC 6539P used in the turbidimetric assay of the amount of active drugs in the intravenous preparations that were administered, DMCT was 90 per cent as active as CTC, 3 times as active as TC, and 1.9 times as active as OTC. In both instances, CTC is seen to be the most rapidly removed from the blood, but its relative activity at different intervals after the dose, as compared with the other antibiotics, appears to be markedly different depending on whether this activity is expressed in terms of the administered drug or compared with a single standard. In these studies, the standard solution of all of the homologues was included in each test.

Such data, although valid, may be somewhat misleading in view of the following considerations: (1) *B. cereus*, the test organism, is not a pathogen and therefore data on "activity" determined in this manner do not necessarily reflect the potency of the individual homologues against organisms encountered in clinical situations, (2) the plasma concentrations may not be adequately adjusted for protein binding, and (3) high blood levels do not necessarily imply high tissue levels in areas invaded by bacteria.

These considerations emphasize a problem that has been repeatedly at the center of the "battle of the blood levels," namely, that activity against specific pathogens is of greater consequence than mere concentration of drug in the serum. This particular facet of the analysis of antibiotic therapy has led some investigators to prefer the broth dilution method of assay for serum, employing an array of common pathogens as test organisms. The latter method, as generally employed, has the disadvantage of using a twofold dilution method which is less accurate than the agar diffusion or turbidimetric methods. It has been repeatedly shown, however, that clinically important differences among drugs can be distinguished by the tube dilution method about as readily as with the agar diffusion assay,[23, 24] and some workers have attempted to reduce the error of this method by using arithmetic dilutions.

Absorption of oral doses

Chelation and the role of excipients. The tetracyclines are incompletely absorbed from the intestines, and fairly large amounts can be recovered in the stools after oral administration.[2-4] Shortly after the introduction of CTC it was found that aluminum hydroxide gels interfere with its absorption, but most workers found that food, milk, and carboxymethylcellulose lacked this effect, and these were actually recommended to reduce the gastric irritation resulting from oral doses.[2] More carefully controlled and detailed studies in rats by Eisner and colleagues[25] indicated enhanced absorption of CTC when given orally with added citric acid. Subsequently,

Dearborn and associates[26] and Sweeney and co-workers,[27] in rats and in humans, respectively, demonstrated a marked depressing effect of dicalcium phosphate on blood levels achieved with oral doses of TC. Price and co-authors[28, 29] noted an antagonistic effect of milk on absorption of OTC and CTC and showed clearly that readily ionizable salts of calcium and magnesium have considerable inhibiting effect on these analogues in vitro. Harcourt and Hamburger[30] demonstrated that concomitant oral administration of magnesium sulfate and TC in man or rhesus monkeys resulted in antibiotic blood levels only about 25 per cent as high as those achieved when the antibiotic was given alone. Oral magnesium sulfate, however, did not affect the blood levels of intramuscularly administered TC.

The chemical basis for the mutual effects of antimicrobial compounds and metallic cations has been extensively reviewed by Weinberg.[31] The tetracycline structure contains numerous sites at which chelation with metallic cations might occur, the most important of these in the lower tier of the molecule which contains essentially two 1,3-diketones with two of the ketones in the enol form. Such monoenols in 1,3-diketones chelate with metallic ions very readily to form six-membered rings. Albert[32] and Albert and Rees[33] observed the formation of drug-metal complexes of 1:1 and, as the pH was raised, of 2:1. The most stable complexes (in order of decreasing stability of drug-metal complexes tested) were Fe^{+++}, Al^{+++}, Cu^{++}, Ni^{++}, Fe^{++}, Co^{++}, Zn^{++}, and Mn^{++}. In addition to metal complexes, TC and OTC also form soluble complexes in aqueous solution with a number of organic compounds which contain strong negative centers, such as sodium salicylate, sodium *p*-hydroxybenzoates, vitamins, and amino acids.[34, 35]

The phenomenon of interference of tetracycline absorption by divalent and trivalent cations has led to extensive efforts on the part of the manufacturers to devise means to counteract this effect. Of the many excipients tried, four have been studied in humans and introduced into therapy: citric

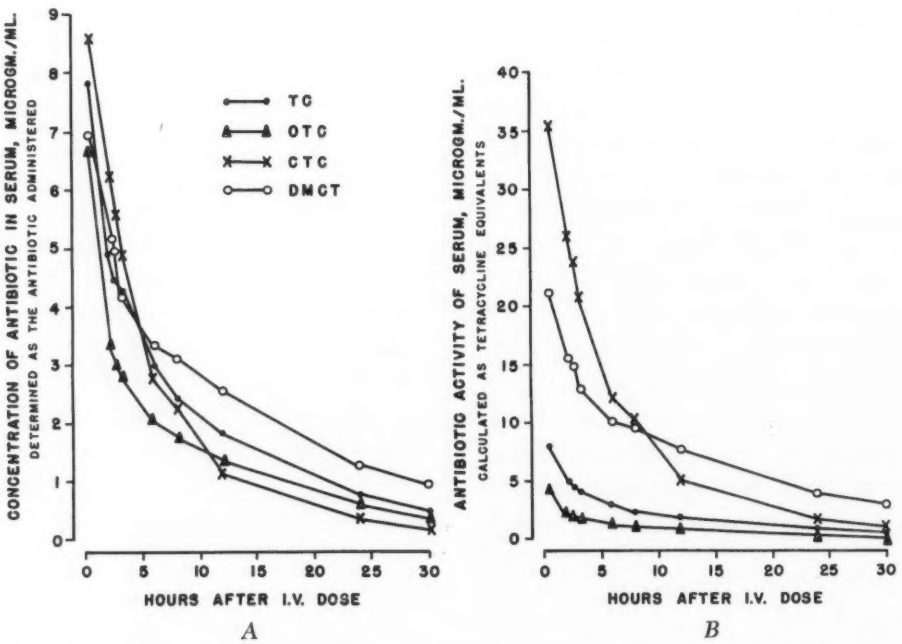


Fig. 2. *A*, The mean levels of four tetracyclines in serum in terms of the antibiotic administered. *B*, The same levels calculated in terms of tetracycline activity (agar diffusion method using *Bacillus cereus*). (From Kunin, Dornbush, and Finland.[20])

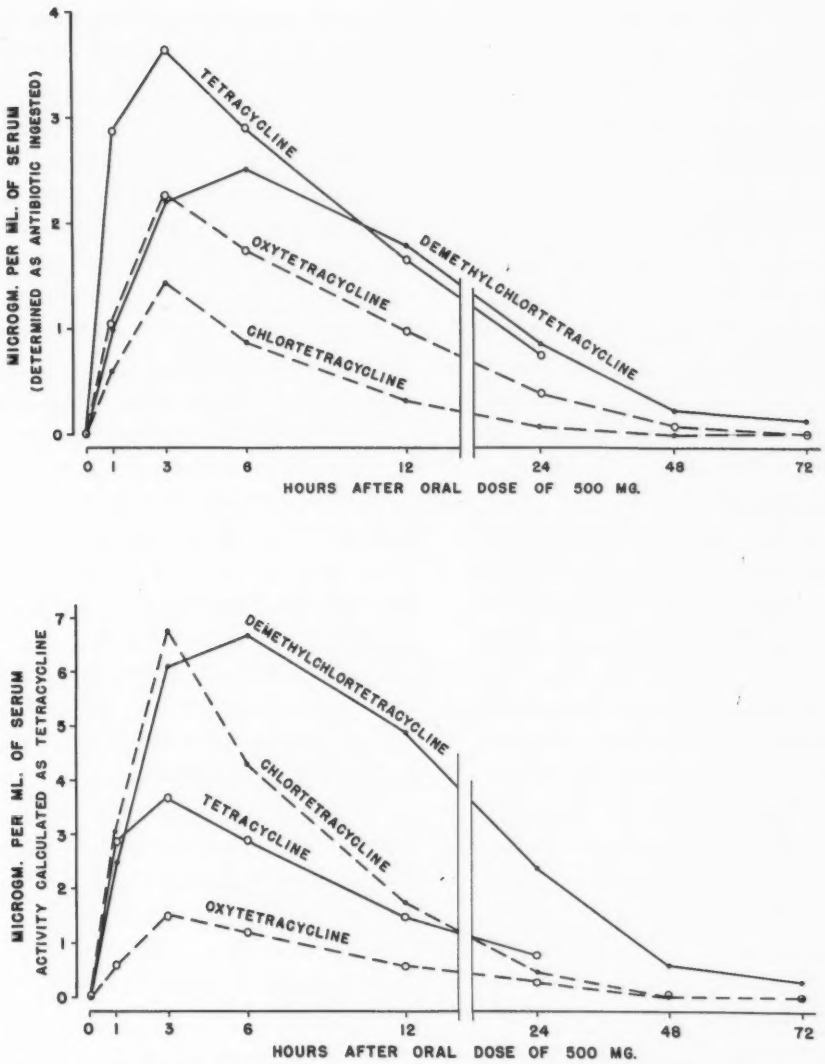


Fig. 3. *A*, The mean concentrations of four tetracycline antibiotics in serum of normal young men after single oral doses of 500 mg. equivalents of their hydrochlorides, the concentrations expressed in terms of weight of the administered homologue. (After Kunin and Finland[21] and Hirsch and Finland.[22]) *B*, The same results calculated in terms of tetracycline activity (agar diffusion method using *B. cereus*).

acid, a phosphate complex of tetracycline, sodium hexametaphosphate, and glucosamine hydrochloride. A critical analysis of their role in antibiotic blood level enhancement has been presented elsewhere.[23, 36] In brief, many of the studies purporting to demonstrate an enhancing effect upon TC absorption were hampered by one or more of the following: (1) the filler in the capsules used contained dicalcium phosphate and some also contained small amounts of magnesium salts,[37] (2) the actual content of the active antibiotic at the time of the study was not stated,[11, 38] (3) data favorable to one or another preparation were inappropriately emphasized,[38, 39] (4) data obtained from heterogenous sources, some uncontrolled and others carefully controlled with crossover studies, were uncritically combined and analyzed,[39] and (5) small, clinically unimportant differences in antibiotic concentration in the blood were given undue emphasis.[38] In other studies in man in which these defects were largely

eliminated,[23, 27, 40, 41] the effects of various excipients, although in some cases statistically significant, were so small as to warrant the conclusion that for therapeutic purposes their addition was of no great importance.

Site of absorption. The most detailed study of the site of absorption of TC is that of Pindell and associates[42] in dogs. They prepared a series of isolated segments of the gastrointestinal tract with intact blood supply. Absorption of TC was determined by serial measurement of the concentration in the blood after instillation into the different segments. The drug was most rapidly absorbed from the duodenum and ileum; peak levels in the blood were reached within ½ hour and were about twice as high from the duodenum as from the ileum. Absorption from the stomach was delayed so that the peak concentration in the blood was achieved after about 4 hours, but it was more sustained than from the other sites. Relatively little was absorbed from the colon. The poor absorption from the colon of dogs is in agreement with earlier studies in man[43, 44] in which CTC and OTC failed to be absorbed when administered by retention enema. The observations of Pindell and associates[42] are supported, in part, by those of Danopoulis and coauthors,[45] who noted that ½ hour after the oral ingestion of OTC in dogs, blood levels were much higher in the left gastric than in the superior mesenteric vein, indicating marked gastric absorption at that time, but the latter authors did not study absorption beyond this brief period.

Pindell and associates concluded that the absorption of TC is a passive phenomenon, dependent directly upon the concentration presented to the intestines at any one time. This conclusion is somewhat surprising in view of the marked differences noted by these workers in the *rate* of absorption of TC from the stomach compared to that from the duodenum or ileum. A possible explanation suggested by the authors is that the intraluminal pH of the various segments of the gastrointestinal tract differs markedly. The tetracyclines are weak bases; for example, the pKa's of OTC hydrochloride are 3.5, 7.6, and 9.2,[7] indicating that at the acid pH of gastric juice, most of it would be in the nonionized form, whereas in the alkaline duodenal fluid, much more would tend to be in the ionized form. Hogben and co-workers[46] have demonstrated that the intestinal mucosa preferentially allows the absorption of the nonionized form of the drug. A more detailed account of the general theory of distribution of drugs across biologic membranes is presented by Milne, Scribner, and Crawford.[47] These considerations may explain in part the more prolonged absorption of TC from the stomach but do not appear to account for the more rapid absorption from the duodenum and ileum.

Individual variations. In a recent study, Sweeney, Dornbush, and Hardy[48] compared the recovery of antibiotic activity in urine and feces after oral and intravenous administration of DMCT. After an intravenous dose, they recovered an average of 52 per cent of the activity in the urine and 6 per cent in the feces during 104 hours of total collections. In 4 subjects previously found to have widely variable serum antibiotic levels after oral doses, they found similar variations in excretion; the one with the highest concentration in the serum excreted 43 per cent of the dose in the urine and 23 per cent in the feces, whereas the one with the lowest concentration in the serum excreted only 9 per cent in the urine and 72 per cent in the feces. In the same individuals, there were only negligible variations in the amounts recovered in urine and feces after intravenous administration. These studies show that the wide differences in serum concentration of DMCT are caused primarily by differences in absorption from the gastrointestinal tract rather than by differences in excretion or metabolism. Similar results may be expected with all of the homologues, although the proportions of each that are recovered in the urine after an intravenous dose vary considerably.[20]

Renal clearance and the role of plasma protein binding

Table I summarizes data reported on the plasma protein binding and renal clearance of the tetracyclines. The considerable variations in protein binding are not surprising. Wozniak[50] recently pointed out that binding of drugs by plasma proteins can be affected by a number of factors, including the concentration of drug in plasma, the protein concentration of the plasma, the species of animal from which the blood is obtained, and the pH, temperature, and buffer employed. The conditions under which the different studies were carried out in the several laboratories varied widely. As shown in Table I, data are available on three different animal species and the assays were performed by three different methods. Nevertheless, the findings in man indicate that CTC is the most highly bound, followed in decreasing order by DMCT, TC, and OTC. The data reported by Wozniak[50] and by Santi and Serembe[52] indicate a similar order of protein binding by canine plasma proteins. Pindell and associates[42] found TC binding in the dog to be almost twice that noted by Wozniak,[50] and Engelund, Terp, and Trolle-Lassen[53] reported almost 60 per cent of this analogue bound to rabbit plasma, but there was very wide variation (32 to 79 per cent) within the data reported by the latter authors.

Plasma protein binding, as determined by the various authors, was taken into account in the calculations of the filterable fraction of the drug available to the kidney, and most of them concluded that the tetracycline analogues are removed by passive glomerular filtration; however, Kaplan, Yuceoglu, and Strauss,[51] working with young children, and Engelund, Terp, and Trolle-Lassen,[53] employing rabbits, presented data suggesting the additional role of active tubular secretion. Probenecid did not increase the clearance of CTC[49] or of TC,[42] and glucosamine did not alter the clearance rate of OTC.[51] Neither the rate of urine flow[20, 49] nor the pH of the urine[53] had any effect on the rate of clearance of the tetracyclines tested. Engelund, Terp, and Trolle-Lassen[53] cite the work of Böttiger,[54] who demonstrated tetracycline fluorescence in the cells of the tubular epithelium as evidence of active tubular secretion; however, the latter worked with rodents and the data may not be entirely applicable to man.

Effect of renal failure on removal of tetracyclines from the body. A number of workers[3, 55-57] have reported accumulation of TC and OTC in the blood of patients with renal failure. Wood and coauthors[56] observed a serum concentration of 80 μg of TC per milliliter in an oliguric patient on the fourth day of therapy. Kunin and colleagues[58] found that the half-life of TC in serum rose from a normal of 8 hours to as long as 57 to 108 hours in anuric individuals, but this prolongation did not occur unless the creatinine clearance was less than 25 ml. per minute or the level of urea or creatinine was elevated in the blood. Unlike TC, however, the half-life of microbially active CTC did not increase much in severe renal failure, so that the latter must be administered in about the same dosage to all patients regardless of the renal status if therapeutic levels are to be maintained in the blood. The nonrenal removal rate of OTC and of DMCT is similar to that of TC, so that these three analogues are handled by uremic patients in the same manner, that is, they tend to accumulate in the blood when there is renal failure. The removal of TC by hemodialysis is restricted; Kunin and colleagues[58] found that it was filtered across the membrane of the artificial kidney at a rate of 21.1 to 38 per cent of creatinine, presumably because of protein binding, among other factors. The restrictions imposed by renal failure on antibiotic therapy have been reviewed elsewhere.[59]

Distribution in the body

The general features of the distribution of CTC, TC, and OTC in the body have been reviewed, respectively, by Lepper,[2]

Table I. *Plasma protein binding and renal clearance of tetracyclines*

	CTC		OTC		TC	
Species	*Percentage bound**	*Percentage of GFR†*	*Percentage bound*	*Percentage of GFR*	*Percentage bound*	*Percentage of GFR*
Human	65-70	38.8				
	48	30	20	85	24	62
	64				31	
			27	142		
Dog			20-35			
					69	33
	59				36	
Rabbit					59	89

*Percentage of antibiotic bound to plasma proteins.

†Percentage of glomerular filtration rate as determined by the ratio of the clearance of the antibiotic to that of either inulin

‡GF = glomerular filtration; TS = tubular secretion.

Dowling,[3] and Musselman.[4] These drugs appear in the milk of lactating patients, pass the placenta into the fetus, and appear in the saliva, cornea, sclera, iris, and vitreous humor. In all reported studies, the concentrations of CTC, OTC, and TC are lower in the cerebrospinal fluid than in the blood, but there are very few comparative studies of the different analogues in this respect except those of Wood and Kipnis,[60] who showed that the best penetration into the cerebrospinal fluid was obtained with TC, the poorest with CTC, that of OTC being intermediate. CTC appears in the bile in a concentration 8 to 16 times that observed in the serum[2]; similar high concentrations have been noted with the other two analogues.[3, 4]

Less information is available concerning the distribution of DMCT. Kunin and Finland[61] demonstrated that, like the other analogues, DMCT is concentrated in the liver and excreted into the bile; they found bile/serum ratios of 2 to 32. Maximum concentrations in the bile after a single intravenous injection were noted at 4 to 7 hours, but concentrations up to 32 times that of the serum were present more than 19 hours following injection. These authors pointed out that the prolonged blood levels noted with DMCT cannot be considered to be due to delayed biliary excretion. Boger and Gavin[62] found small but measurable amounts of DMCT in the cerebrospinal fluid of noninfected patients 4 hours after a single oral dose. The cerebrospinal fluid concentration of this analogue, however, was only about one-twentieth that of the serum at that time. Kohn, Gaskins, and Rall,[63] on the other hand, found the spinal fluid levels of DMCT after 4 to 8 hours of equilibration in the dog to be 17 to 31 per cent of those in plasma obtained simultaneously.

Distribution studies employing fluorescence. The tetracycline antibiotics exhibit a brilliant yellow gold fluorescence when exposed to ultraviolet light at 365 mμ.[64] The bright blue fluorescence of CTC in ultraviolet light (peak at 3,700 Å and secondary at 4,600 Å) has been utilized in assaying this drug in body fluids[65] and has enabled investigators to perform detailed studies of the distribution of these drugs in man and experimental animals.

Helander and Böttiger[66] and Böttiger[54] studied the distribution of OTC and CTC after intramuscular injection in mice. When low doses were used, these drugs were found to be concentrated mostly in the cells of the reticuloendothelial system (bone marrow, spleen, and lymph nodes) and in the liver and kidney. Fluorescence in connective tissue, cartilage, bone, and meninges was not noted unless high doses were employed. Following parenteral injection,

DMCT					
Percentage bound	*Percentage of GFR*	*Assay method*	*Clearance used for comparison*	*Proposed clearance mechanism‡*	*Reference*
		Fluorometry	Inulin	GF	Sirota and Saltzman[49]
41	30	Bioassay	Creatinine	GF	Kunin, Dornbush, and Finland[20]
51		Radioisotope			Wozniak[50]
		Bioassay	Inulin	GF + TS	Kaplan, Yuceoglu, and Strauss[51]
			Creatinine	GF	Santi and Serembe[52]
		Bioassay	Creatinine	GF	Pindell and associates[42]
48		Radioisotope			Wozniak[50]
		Bioassay	Creatinine	GF + TS	Engelund, Terp, and Trolle-Lassen[53]

or creatinine.

both drugs were detected in the intestinal lumen and later in the epithelium of the villi. These authors postulated an enterohepatic circuit whereby OTC and CTC are excreted into the intestine via the bile and then are reabsorbed and recirculated. Their observations are in accord with those of Herrell and Heilman,[67] who measured CTC by microbial assay in the tissues of a patient who died while being treated with this drug and found the concentrations in the kidney and spleen to be 4 times that of the serum, twice as much in the liver, and only one-fourth as much in the cerebrospinal fluid as in the serum.

Titus, Loo, and Rall[68] noted that a fluorescent material localized and persisted in rabbit bone after TC administration. The binding of the fluorophore to bone depended on the pH and was most complete at pH 7. Extracted fluorophore was shown by chromatography and differential ultraviolet spectrophotometry to be identical to anhydrotetracycline. They postulated that binding to metallic cations had occurred in bone, since the fluorescent material could be removed by ethylenediaminetetracetic acid at pH 7.3. These observations have been extended by Rall and co-workers[69] and by McLeay,[70] who reported that CTC, OTC, and TC each appeared in a number of experimental tumors of mice and rats as well as in human tumors. The fluorescence persisted for at least 10 to 20 days in tumor and bone, but not in other tissues. Milch, Rall, and Tobie[64] noted that the induced fluorescence disappeared from all tissues except bone within 6 hours after parenteral injection and persisted in bone for at least 10 weeks. Localization appeared limited to areas of new bone formation, and they presumed that binding occurred to the calcium or matrix of newly formed bone. These findings were confirmed by Häkkinen[71] and by Häkkinen and Hartiala,[72] who also noted that TC accumulated in areas of metastatic calcification induced by the administration of A.T.10 to rats and that calcification was not essential for localization of TC, since it also appeared in fresh wounds and ulcers in dogs.

Two additional studies on fluorescence of TC in tumors have appeared more recently. Vassar, Saunders, and Culling[72a] confirmed the TC-induced fluorescence of human malignant tumors but found it to be confined to histiocytes and debris within the mesenchymal stroma only; malignant cells were found to be nonfluorescent. In addition to persistent fluorescence in bone, they detected temporary localization and persistence in nonspecific skin ulcerations. They also quoted Lacko, Korinek, and Burger,[72b] who found that precipitation of lipoprotein-tetracycline complex occurs in the presence of calcium ions. In the second

study, Phillips and colleagues[72c] reported the finding of fluorescence in 35 patients with cancer who were receiving TC; this phenomenon was confined specifically to lesions having histologic evidence of malignancy. These authors failed to detect fluorescence in benign tumors or inflammatory pathologic conditions. Extraction of TC from the malignant tumors as well as gross observations indicated the concentration of the fluorophor in the cancer tissue to be a direct function of TC dosage. They observed a definite variation in the avidity, or TC-binding capacity, of various histologic types of malignant tumors.

Kohn, Gaskins, and Rall[63] compared the distribution of TC and DMCT in dogs during maintenance of relatively constant plasma levels, as determined fluorometrically. In their studies, DMCT attained higher muscle/plasma ratios (1.6 to 2.5) than did TC (1 to 1.5). In terms of unbound drug in plasma, these values were 5.3 and 2.2 for DMCT and TC, respectively. The observations of Marshall and Strawitz,[73] who studied plasma/muscle ratios of CTC and OTC with a microbial assay, are not completely in accord with those just cited; the latter found the concentration of these analogues in muscle to be only 50 to 80 per cent of that in the plasma. Perhaps the differences can be accounted for, in part, by differences in the assay methods or in animal species employed. More likely, however, the differences are due to the mode of administration of the drugs: Kohn, Gaskins, and Rall[63] studied the distribution after equilibrium had been established, whereas Marshall and Strawitz[73] limited their determinations to concentrations in blood and tissue soon after a rapid intravenous injection. The former method would seem to be preferable since there is at least partial, if not complete, equilibration in patients treated with multiple doses of drugs.

Kohn, Gaskins, and Rall[63] also studied the penetration of TC and DMCT into canine erythrocytes; the erythrocyte/plasma ratios were 0.6 to 1.2 for DMCT and 0.7 to 1 for TC. Watson,[74] employing an in vitro system, human erythrocytes, and a microbiologic assay, found the cells to contain 45 to 50 per cent of the extracellular concentration of TC.

Distribution of radiolabeled tetracyclines. André,[75] employing tritium-labeled TC, studied the distribution of the drug in mice by autoradiography of full animal sections. This method, although more elegant than measurement of autofluorescence,[54, 66] yielded remarkably similar results. André noted that 20 minutes after an injection of labeled TC, the drug was widely distributed in all tissues except brain. The highest concentrations were found in liver (4 times that in blood), kidney (3.3 times that in blood), and spleen (1.8 times that in blood); those in skeletal muscle and myocardium were slightly higher than in blood (1.2 times as much), and thus about the same as noted by Kohn, Gaskins, and Rall[63] but much higher than those reported by Marshall and Strawitz[73] employing different techniques. At 320 minutes, all of the TC had disappeared from the body except from the skeleton, liver, and kidneys. In early abscesses, TC was found in about the same concentration as in surrounding muscle, but it was greatly concentrated in the fibrous capsule around chronic abscesses. Furthermore, André demonstrated penetration of TC into the necrotic tissue of the abscess itself and detected small amounts in the center of the lesion.

Takesue and associates[75a] applied a radiometric method employing tritiated TC for assay of the drug in serum and plasma of laboratory animals. Correlation studies between microbiologic and radiometric assays on tritiated TC in vivo and in vitro in serum and plasma of the rat and dog agreed very satisfactorily. Among the various anticoagulants used in preparation of plasma samples, heparin was the only one that gave plasma concentrations which agreed closely with those found in serum; the others, namely oxalate, citrate, and disodium ethylenediaminetetraacetic acid

(EDTA) lowered the concentrations as compared with serum, presumably because the TC combined with calcium that was already associated with the oxalate, citrate, or EDTA to form a complex which, because of its large molecular size, had a lower solubility in water and might be expected to sediment directly or become occluded to the heavy red blood cells during centrifugation.

Tetracycline, as determined by the autoradiographic technique, appeared to be distributed both intracellularly and extracellularly; the highest concentrations within cells were noted in the white pulp of the spleen, but none could be detected in the circulating formed elements of the blood. Considerable excretion of TC into the bile and then into the intestines was also demonstrated in these studies and in those previously described in mice with the autofluorescence method.[54, 66] Although in man TC and its homologues are also excreted in high concentration into the bile, the enterohepatic cycle may not be as important as the observations in mice would seem to suggest. Glazko, Dill, and Wolf[76] demonstrated that in the rat, the most important route of excretion of chloramphenicol is via the bile, whereas in the human, the renal excretion of this drug is more important.[77, 78]

Snell, Garkuscha and Allen,[79] employing C^{14}-labeled OTC, also found that the liver was the major site of its accumulation (liver to blood ratio 16:1), but the specific activity of the preparation used by these workers was not great enough to yield important information concerning the distribution of OTC in other organs. These studies were extended to man by Leevy, Zinke, and Chey,[80] who determined hepatic concentration by serial liver biopsy and hepatic storage by arterial–hepatic vein differences. In these studies, liver/blood concentration ratios varied from 5 to 419, and in patients with liver disease or intrahepatic shunts, hepatic uptake of the drug was shown to be decreased.

Dunn and colleagues,[81] using I^{131}-activated TC, found that it was sequestered, in general, by fast growing tissues and, in particular, by tumor, liver, and epiphyseal plate of bone. The radioiodinated TC appeared to be inactivated and excreted by the liver.

Relative volume of distribution. Despite wide discrepancies in the actual values of the relative distribution of the tetracyclines reported by various investigators, all agree that the analogues are distributed throughout a volume greater than can be accounted for by the intravascular compartment alone. Spitzy and Hitzenberger[82] reported distribution spaces for TC, CTC, and OTC to be 95, 92, and 90 per cent of the body weight, respectively. Even higher values were obtained by Kunin, Dornbush, and Finland,[20] in a study of the four analogues in healthy young men; their figures were: for CTC 148 per cent, OCT 189 per cent, TC 159 per cent, and DMCT 168 per cent of the body weight.

These data are in accordance with the previously mentioned studies of the distribution of the tetracyclines which demonstrate sequestration of TC in some organs. However, André,[75] working with cats, reported the volume of distribution of TC to be only 30 per cent of the body weight.

Toxicity and untoward effects

The literature on the toxic effects of each of the first three tetracycline homologues, CTC, OTC, and TC, has been rather extensively reviewed and summarized by Florey.[5] The most detailed and critical review of the untoward effects of CTC therapy is contained in Lepper's monograph[2]; Dowling[3] summarized the untoward reactions in 716 TC-treated patients from the literature. Musselman,[4] in his monograph on OTC, however, has chosen to minimize this aspect, emphasizing the negative aspects and the rare clinical report in which a minimum of untoward effects was noted from large doses and his belief, based on his experience, that the gastrointestinal side effects are few and rarely troublesome and that the relative freedom from side

effects is not fully appreciated. Although the rest of that text contains some 664 references, many of them dealing with single or small numbers of case reports, reference is not made to other more extensive and more detailed clinical studies in which serious or numerous complications of OTC therapy were encountered,[83-86] and many papers dealing primarily with studies of toxicity of this drug were not included.[86-90] In some of these studies, OTC has been responsible for the greatest incidence of a "choleriform" type[85] of gastroenteritis and enterocolitis and for a number of deaths in such cases in which *S. aureus* replaced the fecal flora. Thus, Dearing and Heilman[86] reported 44 such cases; 39 occurred in patients under treatment with OTC, and in only 1 had there been treatment with CTC. The frequency with which such severe gastrointestinal complications were encountered in patients under treatment with OTC was much greater than in those treated with CTC,[87] and the incidence was still less frequent in patients treated with TC.[88] It was largely because of this difference that TC rapidly replaced the earlier congeners, at least in the United States.

As these and other reports indicate, the most distressing complications of therapy with the tetracycline group of antibiotics have been nausea, vomiting, and diarrhea, the latter at times severe, particularly when accompanied by overgrowth of resistant strains of *S. aureus* in the feces.[85-87] In general, these side effects have been infrequent and mild when minimum effective doses (usually 1 Gm. daily in divided doses in adults) were used, and their liability to produce diarrhea has been slight with moderate doses. The severe diarrheas, including staphylococcal enteritis, usually subside quite promptly if recognized early and if the drug is discontinued and proper hydration along with antidiarrheal therapy is instituted. These complications are infrequent and generally absent when moderate doses are given intravenously.

The relationship of dosage to the frequency of gastrointestinal complications has also been noted with DMCT. Thus, Perry, Hall, and Kirby[91] observed no untoward effects in 18 patients given 300 mg. twice daily, but nausea and vomiting occurred in 11 (38 per cent) of 29 who received this dose 4 times a day. Lichter and co-workers[92] observed such effects in only 2 of 126 patients given up to 600 mg. daily, in 3 of 12 given 1 Gm., and in 11 of 18 given 2 Gm. a day. In a simultaneous comparison, Finland, Hirsch, and Kunin[93] found that when DMCT was given at 50 or 60 per cent of the dose of TC, the latter caused somewhat more gastrointestinal side effects. Garrod and Waterworth,[94] in a crossover study in 48 normal subjects given 600 mg. DMCT and 1 Gm. TC daily, observed equal frequency and degrees of mild disturbances from both drugs (average 1.4 bowel actions daily from each). These workers also found similar average frequency of bowel actions with the earlier three homologues in a review of the nurses' notes in case records from their hospital.

Although the gastrointestinal complications of therapy with the tetracyclines are the most frequent and so have received the most attention, certain other complications are also of considerable interest. Each of the three earlier analogues has been shown to produce, during prolonged therapy, morphologic and functional changes in the liver,[95-100] a negative nitrogen balance,[98-101] and increased riboflavin excretion into the urine.[98, 101] Elevation of the nonprotein nitrogen level in the blood during OTC therapy has also been reported,[84, 102] and in rabbits given large doses of tetracyclines, Farhat, Schelhart, and Musselman[103] demonstrated rising nonprotein nitrogen in the blood, anorexia, weight loss, lethargy, convulsions, and respiratory failure.

One may speculate about the biochemical mechanisms responsible for the remarkable effects of the tetracyclines on nitrogen and riboflavin metabolism in man on the basis of the marked concentration of these drugs within the liver, their chelating properties, and the ability of riboflavin,

under certain circumstances, to reverse its antimicrobial activity.[104] CTC has been shown to inhibit oxidative phosphorylation in mitochondria of animal tissues,[105-107] and, under proper conditions, this can be reversed by Mg^{++}.[107] CTC also inhibits organic nitroreductase in cell-free extracts,[108] and this action is reversed by manganese.[109] Inhibition of blood coagulation by the tetracyclines has been considered as possibly the result of the binding of calcium.[110, 111]

Another indirect effect of tetracycline therapy, peptic ulceration and bleeding in uremic patients, has been reported by Lieber and Desneaux.[112] This effect was studied in detail by Lieber and Lefèvre,[113] who found the low level of free and total acid in the gastric juice of uremic patients to be associated with high gastric ammonia content; when OTC (or other antibiotics) was administered, the gastric ammonia was stoichiometrically replaced by urea, presumably due to suppression of formation of bacterial urease in the gastric mucosa, resulting in an increase in gastric acid. Thus, the protective effect upon the gastric mucosa afforded by ammonia released from high concentrations of urea in the uremic patient was abolished by antibiotic therapy and, in some cases, resulted in hemorrhage and ulceration.

Drug fevers and various types of rashes have been observed with each of the tetracycline drugs but have been relatively infrequent, and there have been essentially no blood dyscrasias clearly attributable to them.[5] Pruritus ani and vulvae have been encountered with varying frequency in some groups of patients. As with all antibiotics, particularly those with a wide spectrum, superinfections caused by overgrowth of resistant organisms, including staphylococci, enteric bacteria, yeasts, and fungi, have been reported with varying frequency. They are particularly frequent and severe in hospitalized patients who are treated for long periods or with large doses and in patients with other serious and debilitating diseases, especially those who are receiving therapy with other toxic agents such as radiation, antitumor drugs, and adrenocortical hormones. Strains of Candida, in particular, are frequently increased in the feces during treatment with tetracyclines, and their numbers can be reduced by concommitant administration of nystatin or amphotericin B, but their clinical significance in most cases is questionable. Nutritional deficiencies, some of specific types and allegedly reversible or preventable by administration of the specific vitamins, have also been noted, and these also are difficult to assess.

Of particular interest recently has been the occurrence of an exaggerated sunburn reaction resulting from direct exposure of patients to sunlight during therapy with moderate or large doses of DMCT; such reactions have been only rarely noted with the other homologues. Shaffer and associates[114] reported this reaction during CTC treatment of patients with chronic liver disease. Morris[115] observed an ambulatory patient at the seaside resort of Corpus Christi, Texas, who developed a severe sunburn of exposed surfaces with marked local and systemic symptoms after an early afternoon exposure to bright sunlight early in September, on the fifth day of treatment with 600 mg. of DMCT daily. This was accompanied by high fever, eosinophilia, and increased blood platelets. This reaction was reproduced in a much milder form by readministration of the same drug 2 months later. Four other instances were reported by Falk[116] from among 27 ambulatory patients seen in Reno, Nevada.

This unusual reaction has been the subject of intensive study by a number of competent investigators. It was reproduced quite regularly in Philadelphia early in September by proper exposures to bright, midday sunlight in volunteers receiving adequate oral doses (600 mg. daily or more) but could not be reproduced with 1 or 2 Gm. daily of TC for 4 days.* Carey[117] was able to uncover a total of 40

*A. M. Kligman: Personal communication, Oct. 6, 1959.

patients among 2,682 treated with DMCT who exhibited this reaction or anything resembling it. The reaction occurs only after systemic administration on skin exposed directly to sunlight containing rays in the range 2,700 to 3,200 Å; these are filtered out by ordinary glass and are encountered in temperature zones only in the summer. It cannot be reproduced by direct exposure of skin to which the DMCT is applied topically. The reaction is one of pure phototoxicity and not one of hypersensitivity, an important distinction as recently noted by Harber, Lashinsky, and Baer[118] with respect to chlorothiazide. The exact basis of this reaction is not clear and is now under intensive study.

Therapeutic effects

For a survey of the clinical uses and effectiveness of the tetracycline antibiotics, the reader is referred to the monographs and reviews mentioned in the opening paragraph, particularly to the one by Florey.[5] Although, as already noted, individual species and even individual strains of bacterial pathogens may be more susceptible to one or another of these homologues, the similarity of their action is far greater than any differences they may exhibit, and the differences are quantitative rather than qualitative. Moreover, any attempt to make a broad comparison of the results of therapy with the different analogues or even with other antibiotics, particularly chloramphenicol, would be foolhardy because there are too few studies in which more than one of these drugs was used and in which their effects were properly observed at the same time, by the same observers, under similar conditions, and in a sufficiently large number of comparable cases to permit any meaningful comparisons. In two limited studies carried out at the Boston City Hospital in which such comparisons were attempted, no differences in therapeutic effectiveness could be discerned between OTC and CTC used in similar doses[87] or between TC and DMCT, the latter used at 50 to 60 per cent of the dosage of the former.[93] The differences observed in the untoward gastrointestinal effects during these studies have already been discussed.

One of the most important aspects of therapy with this group of antibiotics has been the increase in incidence of pathogenic bacteria, notably staphylococci and enteric bacteria, that are resistant to them in vitro. This is also accompanied by failures of therapy in infections caused by these resistant organisms. All of the homologues are involved in this phenomenon, although resistance of some species may be greater to one or another of the homologues, as illustrated among the staphylococci studied at the Boston City Hospital. Strains isolated there in 1951-1952 were found to be more susceptible to CTC than to OTC[119]; among those studied in the same hospital in 1958,[120] the sensitive strains were found to be quantitatively more susceptible to CTC and DMCT than to TC or OTC, whereas a rather high degree of resistance was most frequent to OTC, somewhat less to CTC and DMCT, and least to TC. In the latter study, demethyltetracycline, which has not been introduced into clinical use, was found to be the least active against sensitive strains and a greater proportion was resistant and to a higher degree. In vitro, also, there is complete cross resistance to the other homologues when bacteria are rendered resistant by repeated subcultures in sublethal concentrations of any one of them; this has been clearly demonstrated for the first three homologues,[121] but is undoubtedly also true of DMCT, and is also true of resistant organisms freshly isolated from patients, although the degree of their resistance to the individual homologues may differ somewhat as illustrated among the staphylococci just mentioned.

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Next comes the status of pharmacology in American medical education. Relative to the value of therapeutic discoveries in the past thirty years, and relative to the potentialities of chemotherapy, and relative to the size of the drug industry in the United States, the support and esteem "enjoyed" by pharmacology in the medical curriculum is absurd. And the scarcity today of pharmacologists qualified for teaching posts, governmental appointments or industrial positions is lamentable—or exciting, if you don't think it is too late.

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BY ALAN GREGG, JOURNAL OF THE AMERICAN
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Laboratory and clinical studies of penicillin X-1497

Laboratory and clinical studies of a new penicillinase-resistant penicillin, X-1497, showed it to be active against coagulase-positive staphylococci resistant to penicillin G. All fifty strains tested were inhibited by 5 μg per milliliter or less of X-1497. Staphylococcal killing with X-1497 was comparable to that with vancomycin and bacitracin. Serum from subjects receiving X-1497 was active against penicillinase-producing staphylococci. Average peak serum levels were 9.6, 14.6, and 24 μg per milliliter after 0.5, 1.0, and 1.5 Gm. intramuscular injections, respectively. The results of treatment in 23 patients were generally favorable, including 11 cases of staphylococcal infection. The drug was remarkably nontoxic. Although X-1497 has the disadvantage of requiring frequent intramuscular injections to maintain adequate antibiotic blood levels, it promises to be a safe and highly effective antistaphylococcal agent.

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The observation of Batchelor and associates[1] that 6-aminopenicillanic acid could be obtained directly by fermentation has led to the synthesis of a large number of new penicillins. One of these compounds, penicillin X-1497,† appeared in preliminary studies to be remarkably resistant to destruction by penicillinase and to be nontoxic and readily absorbed following intramuscular administration.* Our preliminary laboratory and clinical studies of this promising antibiotic will be presented in the present report.

In vitro studies

Susceptibility tests. Concentrations of penicillin X-1497 and penicillin G that cause inhibition of growth of group A streptococci and pneumococci were determined in tryptose phosphate broth containing 1.5 per cent human blood. After overnight incubation, the lowest concentration of the antibiotic causing complete inhibition of growth, by gross inspection, was recorded. A similar method was used for staphylococci, except that blood was not

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†Since writing this report, X-1497 has become known to us as sodium 2,6-dimethoxyphenyl penicillin. The trade name of Bristol Laboratories Inc. for this antibiotic is Staphcillin.

*E. M. E. Morigi: Personal communication.

Table I. *Susceptibility of pneumococci and group A streptococci to X-1497 and penicillin G*

Organism	*Number of strains*	*Minimal inhibitory concentration*	
		X-1497 (μg per ml.)	*Penicillin G (U. per ml.)*
Pneumococci	3	0.25	0.05
	3	0.125	0.05
	2	0.062	0.025
	1	0.125	0.025
	1	0.125	0.012
	Total 10	Average 0.15	Average 0.04
Group A streptococci	7	0.125	0.012
	2	0.25	0.025
	1	0.25	0.012
	Total 10	Average 0.16	Average 0.015

added to the broth. In all instances, 0.5 ml. amounts of a 10^{-2} dilution of an overnight culture were added to equal amounts of broth containing appropriate concentrations of the antibiotic.

The results are presented in Tables I and II. The data show that with pneumococci, X-1497 was about one-fourth as active as penicillin G, and with group A streptococci, it was on the average one-tenth as active. Because of the small number of strains and the twofold dilutions employed, these differences between streptococci and pneumococci are probably not significant. Fifty strains of recently isolated coagulase-positive staphylococci were inhibited by 5 μg per milliliter or less of X-1497 despite the fact that all were resistant to 100 U. per milliliter of penicillin G and were penicillinase producers. The average minimal inhibitory concentration of X-1497 with these fifty strains was 3.8 μg per milliliter, only slightly higher than with thirty penicillin-sensitive staphylococci (2.8 μg per milliliter). The ability of the fifty resistant strains to produce penicillinase had little influence on their susceptibility to X-1497, although they did require more of the drug to cause inhibition.

Bactericidal studies. The killing action of X-1497 was compared with penicillin G, vancomycin, and bacitracin in simultaneous tests against phage type 80/81 staphylococci. For the comparisons with vancomycin and bacitracin, an organism resistant to 100 U. per milliliter of penicillin G was used, whereas comparisons with penicillin G employed a staphylococcus resistant only to concentrations below 0.25 U. per milliliter of the latter drug. To perform the tests, a 10^{-2} or 10^{-3} dilution of an 18 hour broth culture of the test organism was made in a 250 ml. screw-capped bottle. After thorough mixing, 18 ml. aliquots were distributed in three 125 ml. flasks. Aqueous solutions of X-1497 and of penicillin G, bacitracin, or vancomycin were then added to two of the flasks to give the desired concentrations of antibiotic, and an appro-

Table II. *Susceptibility of coagulase-positive staphylococci to penicillin X-1497*

Staphylococci	*Number of strains*	*Minimal inhibitory concentration of X-1497 (μg per ml.)*
Penicillin-susceptible (M.I.C. < 0.5 U. per ml.)	23	2.0
	8	5.0
	Total 31	Average 2.8
Penicillin-resistant (M.I.C. > 100 U. per ml.)	20	2.0
	30	5.0
	Total 50	Average 3.8

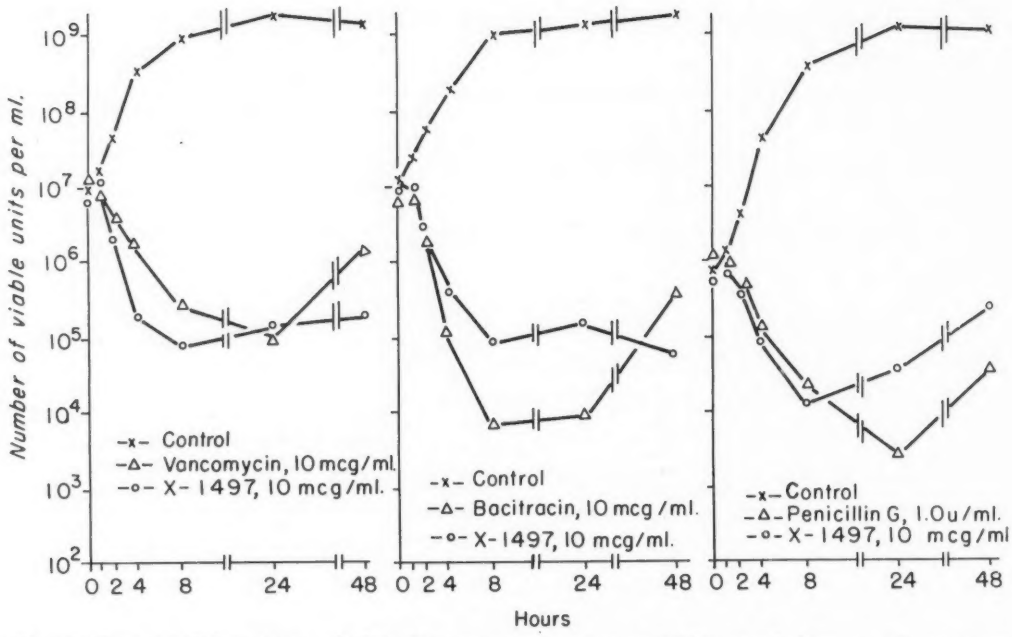

Fig. 1. Bactericidal activity of X-1497 against phage type 80/81 staphylococci. Comparisons with vancomycin and bacitracin employed a strain resistant to 100 U. per milliliter of penicillin G, whereas in the comparisons with penicillin G, a strain resistant only to concentrations below 0.25 U. per milliliter was used.

priate amount of distilled water was added to the third (control) flask. The contents of each flask were then distributed in 1 ml. amounts to a series of eight 25 by 150 mm. test tubes. To perform plate counts, appropriate dilutions of one tube from each series were made in saline immediately and after 1, 2, 4, 8, 24, and 48 hours. A dilution of at least 10^{-3} was made in all instances to minimize the influence of the antibiotic present in the undiluted tubes. After the dilutions were mixed in warmed agar in Petri dishes, the agar was allowed to harden, and the number of viable units were counted after incubation for 24 and 48 hours for each sample.

The results are depicted in Fig. 1, where each point on the curves represents the average of two determinations done on different days. The concentrations of antibiotics used to produce these killing curves were 10 μg per milliliter for X-1497 and vancomycin, 10 U. per milliliter for bacitracin, and 1 U. per milliliter for penicillin G. In each instance, the amount selected was 2 to 4 times that which usually gave inhibition in determinations of the minimal inhibitory concentration. X-1497 killed staphylococci at a rate slightly greater than vancomycin, but not so great as bacitracin. Of the three antibiotics, regrowth of persisting organisms occurred the least with X-1497. When X-1497 was compared with penicillin G against susceptible staphylococci, the rates of killing were approximately equal for the two drugs over the first 8 hours. Thereafter, the regrowth of persisting organisms was delayed by penicillin G as compared with X-1497.

These findings suggest that the bactericidal activity of X-1497 compares favorably with three antistaphylococcal antibiotics of proved effectiveness, namely, vancomycin, bacitracin, and penicillin G.

Lysis of staphylococci. The lytic action of X-1497 against coagulase-positive staphylococci was compared with that of penicillin G, bacitracin, and vancomycin using several concentrations of each antibiotic. Cuvettes (25 by 105 mm.) containing the

various concentrations of drug in 8.5 ml. of tryptose phosphate broth were placed in a 37° C. water bath and inoculated with 1.5 ml. of a 13 to 14 hour broth culture, giving an optical density of approximately 0.10 with the Coleman spectrophotometer (wave length 575 mμ). Readings of optical density were than made at 1, 2, 4, and 24 hours, or more often in some instances.

The results are presented graphically in Figs. 2 and 3 and show that with penicillin G, 0.1 U. per milliliter, and a sensitive staphylococcus, there was a dramatic rise in optical density over the first 4 hours, followed by a marked decrease in turbidity as the staphylococci underwent lysis. X-1497, 2 μg per milliliter, produced striking lysis of penicillin-resistant staphylococci as well as of those which were penicillin sensitive, whereas lysis was completely lacking with bacitracin, 2 U. per milliliter, or vancomycin, 4 μg per milliliter. The results were variable from day to day, but the figures provide representative examples of what was observed.

The significance of these observations remains to be determined. The marked rise in optical density preceding lysis caused by low concentrations of penicillin G is known[2] to be associated with a marked swelling of the staphylococci. It is possible that this phenomenon is related to the interference by penicillin G with bacterial cell wall synthesis and that X-1497 may have a similar mode of action.

Development of resistance. The development of resistance of coagulase-positive staphylococci to X-1497 was studied in comparison with penicillin G and vancomycin. The amount of 0.5 ml. of a 10^{-2} dilution of an overnight culture in tryptose phosphate broth was added to a series of tubes containing 0.5 ml. of antibiotic solution decreasing by twofold in concentration. After 24 hours incubation at 37° C., the tube with the highest concentration of antibiotic showing growth of the test staphylococcus was subcultured in broth. This subculture was incubated 24 hours at 37° C. and then used in 10^{-2} dilution as inoculum for a second serial twofold dilution test in fresh antibiotic solutions. Following incubation, the process of subculturing and inoculating tubes containing serial dilutions of the antibiotic was continued at least ten times. Tests with the two antibiotics being

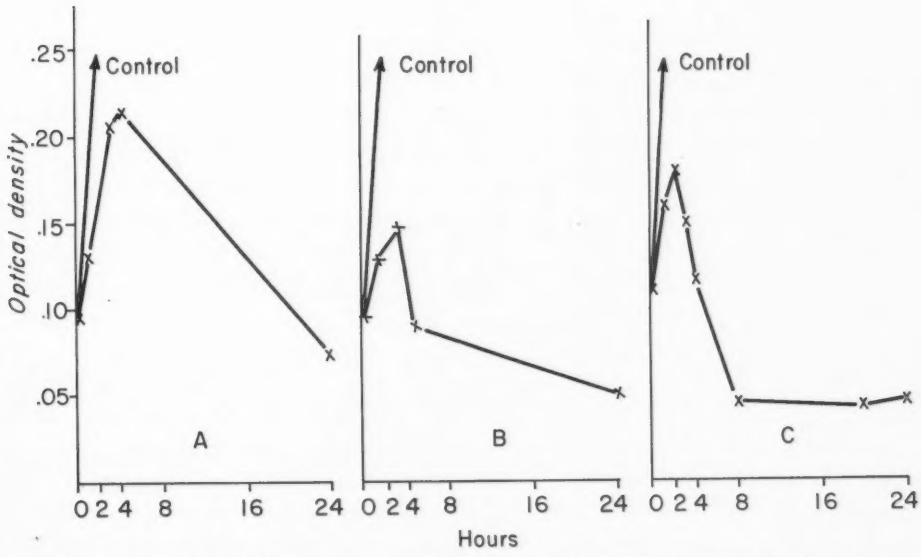

Fig. 2. Lysis of staphylococci by penicillin G and X-1497. *A*, Penicillin G, 0.1 U. per milliliter, sensitive staphylococcus. *B*, X-1497, 2 μg per milliliter, penicillin-sensitive staphylococcus. *C*, X-1497, 2 μg per milliliter, penicillin-resistant staphylococcus.

compared were carried out simultaneously, and each test of susceptibility of the strain developing resistance was accompanied by a determination of the susceptibility of the parent strain. A phage type 80/81 staphylococcus resistant only to concentrations of penicillin G less than 0.25 U. per milliliter was used for the comparison of X-1497 with penicillin G; the comparison of X-1497 with vancomycin utilized a strain of phage type 80/81 resistant to 100 U. per milliliter of penicillin G.

The results are presented graphically in Fig. 4 and show that with these strains of staphylococci, X-1497 resistance developed somewhat more slowly than resistance to penicillin G and only moderately faster than with vancomycin.

Development of resistance to penicillin G during therapy of a susceptible staphylococcal infection has not been shown to occur in patients, and the same may prove true with X-1497. In 2 patients infected with coagulase-positive staphylococci, 1 with osteomyelitis and 1 with empyema, we were able to isolate staphylococci from the lesions on the eleventh day of therapy with X-1497, 4 to 6 Gm. daily. Tube dilution susceptibility tests done with these strains side by side with earlier isolates from the patients showed no change in susceptibility to X-1497.

Resistance to penicillinase. Amounts of 1 ml. of an 18 hour culture of a penicillinase-producing staphylococcus were added to tubes containing 9 ml. of tryptose phosphate broth to make a final concentration of 2 μg per milliliter of X-1497. After 1, 4, and 8 hours, representative tubes were heated for 30 seconds at 80° C., and the contents were passed through a Swinney filter. The filtrate was then assayed for antibiotic activity by the tube dilution method using a group A streptococcus as test organism. A similar study was carried out simultaneously with penicillin G, 10 U. per milliliter. Appropriate control tubes containing antibiotic solutions without the organism and inoculated solutions without antibiotic were employed.

Under the conditions of this study, there was no detectable destruction of X-1497 by the staphylococcus, although penicillin G under the same conditions was completely inactivated by the large amounts of penicillinase present.

Sera from patients and volunteers who received X-1497 were tested for activity against coagulase-positive staphylococci resistant to 100 U. per milliliter of penicillin G. The results of these assays gave essentially the same end points as when similar tests were performed with staphylococci sensitive to 0.2 U. per milliliter or less of penicillin G, thus indicating that X-1497 entered the serum in a form resistant to destruction by staphylococcal penicillinase.

The effect of inoculum size on the minimal inhibitory concentration of X-1497 for a penicillinase-producing staphylococcus was also studied. With inocula varying

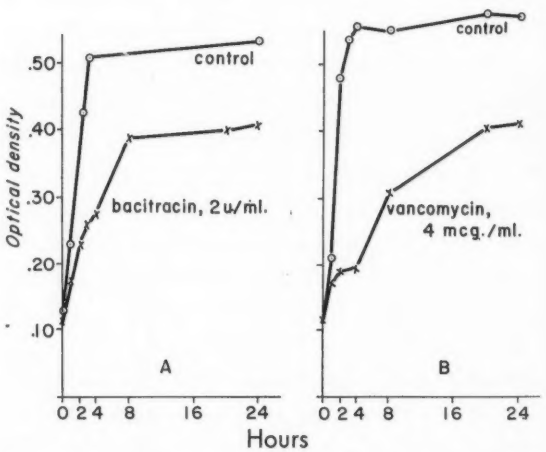

Fig. 3. Lack of lysis with: *A*, Bacitracin, 2 U. per milliliter. *B*, Vancomycin, 4 μg per milliliter. Penicillin-resistant staphylococcus.

from 0.5 ml. of a 10^{-4} dilution to 0.5 ml. of a 10^{-1} dilution of an overnight culture, there was only a twofold (one tube) rise in minimal inhibitory concentration, identical to that in a simultaneous test with vancomycin. This was corroborative evidence that staphylococcal penicillinase has little, if any, effect on the susceptibility of staphy-

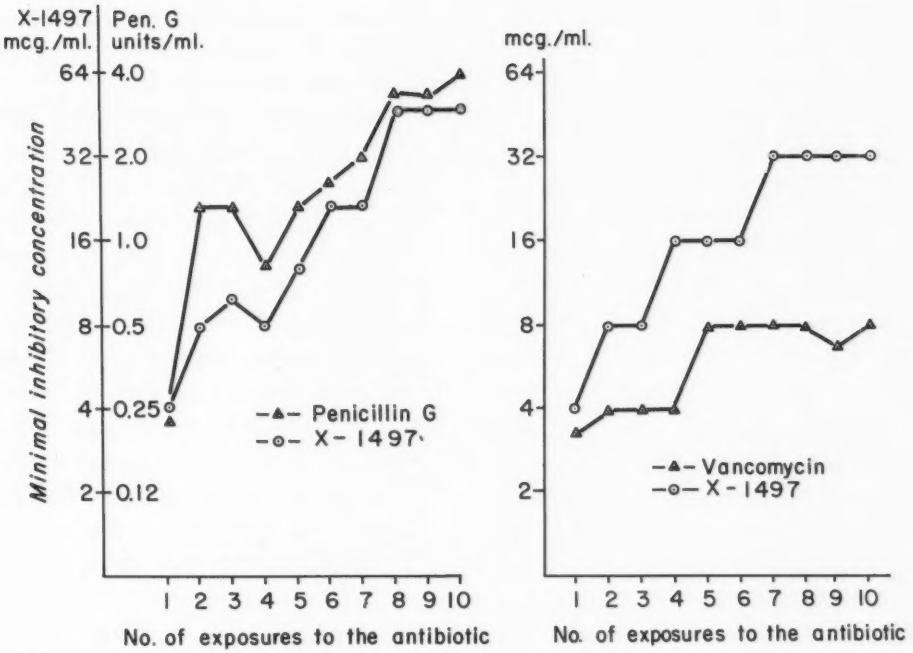

Fig. 4. Development of resistance of phage type 80/81 staphylococci: X-1497 compared with penicillin G and vancomycin.

lococci to X-1497. In contrast, there is a tremendous effect of inoculum size when penicillinase-producing staphylococci are tested against penicillin G,[3] the minimal inhibitory concentration being altered from 2 U. per milliliter to greater than 100.

Effect of serum on minimal inhibitory concentrations. The effect of normal human serum in concentrations from 6.25 per cent to 100 per cent was tested with a group A streptococcus and a coagulase-positive staphylococcus. It was found that with concentrations of serum up to 25 per cent, there was no effect on the minimal inhibitory concentrations. Higher concentrations resulted in a one tube (twofold) increase in the amount of antibiotic required to inhibit growth. Identical results were obtained in simultaneous tests with penicillin G.

Stability of X-1497. Tryptose phosphate broth (pH 7.3) containing 50 μg per milliliter of X-1497 was distributed in 5 ml. amounts to screw-capped tubes. One tube was immediately refrigerated at –20° C., and the remaining tubes were incubated at 37° C. At each 24 hour interval for 8 days, a tube was taken from the incubator and stored at –20°. All of the refrigerated tubes were then allowed to thaw and were assayed for antistreptococcal activity with a tube dilution method. A similar study was carried out simultaneously with penicillin G, 5 U. per milliliter. In order to be sure that the remaining active antibiotic was resistant to staphylococcal penicillinase, the contents of the tubes from the X-1497 series were also assayed with a naturally resistant coagulase-positive staphylococcus.

The results of these studies indicated that X-1497 was at least as stable in broth at 37° C. as penicillin G, if not more so. X-1497 activity declined 1.5 twofold dilutions, as opposed to 3 twofold dilutions for penicillin G (averages of two determinations). Moreover, deterioration of X-1497 was essentially the same when the resistant staphylococcus was used for assay, showing that the active antibiotic present was effective against penicillinase-producing staphylococci.

Table III. *Serum concentrations (μg per ml.) of X-1497 after a single intragluteal injection (averages for 6 volunteers)*

Time (hr.)	Dose (Gm.)		
	0.5	1	1.5
½	9.67	12.67	18.67
1	8.00	14.67	24.00
2	4.33	10.00	16.00
4	0.17	1.92	2.5

Table IV. *Serum concentrations (μg per ml.) of X-1497 after an intravenous infusion of 1 Gm. over a 30 minute period in 100 ml. normal saline (averages of 2 volunteers)*

Time (hr.)	Without probenecid	With probenecid
0	40	60
½	10	30
1	5	15
2	1	6
4	< 0.5	1.5
6	< 0.5	< 0.5

Pharmacologic studies in man

Intramuscular X-1497. Serum levels of X-1497 were determined in 6 volunteers after intragluteal injections of 0.5, 1, and 1.5 Gm. The injected volume was 2 ml. in all instances, and the diluent was sterile water for injection. Blood specimens were obtained ½, 1, 2, and 4 hours after injection. The clot was allowed to retract at room temperature, and the serum was separated and stored at –20° C. After all specimens had been collected, they were assayed by a serial twofold tube dilution method employing a group A streptococcus as the test organism. All serum specimens from any one person were assayed at the same time.

The results are presented in Table III, where each number represents the average serum concentration in micrograms per milliliter attained in 6 volunteers. The peak levels with 0.5, 1, and 1.5 Gm. were 9.6, 14.6, and 24 μg per milliliter, respectively. There was then a rapid decline, with levels scarcely in the therapeutic range at 4 hours, even with the larger doses.

Intravenous X-1497. Serum levels of X-1497 after a 30 minute intravenous infusion of 1 Gm. in 100 ml. of normal saline were studied in 2 volunteers. Blood specimens were obtained ½, 1, 2, 4, and 6 hours after the start of the infusion. After a 3 day interval, the study was repeated while the subjects were receiving probenecid, 0.5 Gm. being given orally 2 hours before the start of the infusion and again 3 hours after the infusion. Serum specimens were treated as with the intramuscular study, assays of all sera from the same person being done simultaneously.

The results with intravenous administration are presented in Table IV. Each number represents the average of results from 2 volunteers. Without probenecid, the peak level was 40 μg per milliliter, falling rapidly so there was no detectable antibiotic at 4 hours. With probenecid, the levels were substantially higher and more prolonged, the peak being 60 μg per milliliter. Even with probenecid, the drug was excreted so rapidly that little remained after 4 hours.

Clinical results

Penicillin X-1497 was supplied in 0.5 Gm. ampules in powder form which went rapidly into solution when 0.6 to 1 ml. of sterile water was added. Most patients were treated by combining the contents of two ampules and injecting the 2 ml. volume intramuscularly four to six times daily. Some patients received intravenous X-1497 in doses of 1 Gm. four to six times daily. To prevent irritation to the injected vein, the drug was diluted in 10 to 15 ml. of normal saline and injected over a 5 minute period, either through a syringe or into the tubing if an infusion was running. The initial dose was usually 6 Gm. daily, and this was reduced to 4 Gm. as soon as there were signs of clinical improvement, usually after 24 to 48 hours. Patients with severe staphylococcal infections received 6 Gm.

daily for a longer period. Only 1 patient was treated with a larger dose, namely, 3 ampules (1.5 Gm.) intramuscularly four times daily. A summary of the 23 patients treated, with their response to therapy, is presented in Table V.

Pulmonary infections. These 9 patients with pneumonia were adults, mostly in the older age groups, or were younger, debilitated alcoholics. Patients with staphylococcal pneumonia were not included under this heading. In the 7 patients who were considered to have been "cured," there was definite decrease in fever, toxicity, cough, and sputum during the first 48 hours. Six were afebrile within 5 days, although 1 debilitated alcoholic had fever for 12 days, an occurrence we have observed frequently in the past in alcoholics treated with penicillin G. All these patients received the drug for 5 days only, in doses of 4 or 6 Gm. daily. Past experience has demonstrated that, for most patients, 5 days of penicillin therapy is adequate in pneumococcal pneumonia.

The patient listed as having undiagnosed pneumonia presumably had a Gram-negative infection which did not respond to 8 days of treatment with X-1497 or to streptomycin, chloramphenicol, or tetracycline. Clinical results in these 9 patients with pneumonia were felt to be comparable to those obtained with penicillin G therapy.

Staphylococcal infections. Three patients with staphylococcal pneumonia were treated with 8 to 10 day courses of penicillin X-1497, and 2 appeared definitely improved. The third patient was an old man who remained febrile through 8 days of therapy and developed a lung abscess in the area of pneumonitis. This may have been an aspiration lung abscess, although staphylococci were cultured from the tracheal aspirate. Fever promptly disappeared, and resolution of the lung abscess followed change to chloramphenicol therapy.

Results were quite impressive in the 2 patients with acute osteomyelitis. One patient, a 14-year-old boy, had high spiking fever and severe pain in the left sacroiliac region, with two blood cultures positive for staphylococci. His temperature gradually fell to normal during the first week of therapy, and the pain subsided almost completely. When the drug was discontinued

Table V. *Summary of clinical results obtained with penicillin X-1497*

Clinical problem	*Cured*	*Improved*	*Indeterminate or unimproved*	*Total*
Pulmonary infections				
Bacterial pneumonia	7			7
Tuberculous pneumonia			1	1
Undiagnosed pneumonia			1	1
Staphylococcal infections				
Pneumonia		2	1	3
Acute osteomyelitis*		2		2
Chronic osteomyelitis		2		2
Empyema			1	1
Septicemia		1		1
Soft tissue infections		2		2
Miscellaneous infections				
Streptococcal cellulitis	2			2
Cat-scratch disease			1	1
				23

*One of these 2 patients had two blood cultures positive for *Staphylococcus aureus* prior to therapy.

after 21 days, there were still no bone changes apparent on x-ray, and he had moderate pain on walking. The other patient, a woman with acute osteomyelitis of the tibia, showed marked relief of pain and redness after receiving the drug 3 or 4 days. However, an abscess appeared in the soft tissues next to a surgical incision, and this drained and contained staphylococci throughout her 16-day course of therapy. A month later, there was still drainage and a recurrence of bone pain. In this instance, more prolonged therapy might have given a better result. One patient with chronic osteomyelitis of the hip responded well to surgical removal of dead bone and to drug therapy, but the exact value of the drug in this case is difficult to assess. The other patient with chronic osteomyelitis showed a decrease in inflammation and drainage prior to surgical operation, and the results since then are not yet known. A patient with furuncles and one with a carbuncle responded well.

The patient with septicemia was a young child with acute leukemia for whom blood cultures became negative and whose fever declined during therapy. His condition continued to deteriorate because of his underlying illness.

Miscellaneous infections. The 2 patients with streptococcal cellulitis both had widespread involvement of the lower legs, and both became afebrile within 6 days on therapy with 4 Gm. of X-1497 daily.

Side effects. There was remarkable freedom from side effects. Patients appeared able to tolerate a 2 ml. injection containing 1 or 1.5 Gm. of X-1497 with minimal discomfort consisting of pain in the muscle, which usually subsided within 10 minutes. One patient, a 50-year-old physician, received more than seventy injections in the deltoid muscles over a 14 day period with only minimal complaints and with mild induration in the injection sites which cleared within 48 hours. A 14-year-old boy received virtually all of his 120 injections of 1 Gm. each of X-1497 in the right buttock over a 21 day course of therapy without complaint and with only mild induration at the site of injection.

Two patients complained of mild anorexia and a persistent "salty" taste during intravenous therapy, but this did not necessitate stopping the drug. One patient developed peeling of the skin on both hands after 2 weeks of therapy, but the exact relationship of this to the medication was difficult to define.

Summary and conclusions

Penicillin X-1497 was active against penicillinase-producing staphylococci, all fifty strains tested being inhibited by 5 μg per milliliter or less. Strains sensitive to penicillin G were slightly more susceptible (average M.I.C. 2.8) than were penicillinase producers (3.8). Activity against pneumococci and group A streptococci was one-fourth to one-tenth that of penicillin G. The drug was bactericidal, giving killing comparable to penicillin G, vancomycin, and bacitracin against coagulase-positive staphylococci. X-1497 produced lysis of staphylococci in low concentrations, similar to the effect of penicillin G. Strains of phage type 80/81 staphylococci developed resistance to X-1497 somewhat more slowly than to penicillin G, but moderately faster than to vancomycin. Penicillin X-1497 was resistant to destruction by penicillinase-producing staphylococci even when incubated with large inocula for a period of 8 hours, and large variations in inoculum size had little effect. Sera from subjects receiving X-1497 were active against penicillinase-producing staphylococci, indicating that the antibiotic was absorbed in a form resistant to penicillinase. Human serum had little effect (one twofold dilution, the same as with penicillin G) on the amount of drug needed to give bacterial inhibition. X-1497 was at least as stable as penicillin G when incubated in tryptose phosphate medium at 37° C.

Serum levels of X-1497 after intragluteal injections of 0.5, 1, and 1.5 Gm. showed peaks of 9.6, 14.6, and 24 μg per milliliter, respectively (averages of results from 6

volunteers). Intravenous infusion of 1 Gm. of X-1497 in 100 ml. of saline over 30 minutes produced a peak of 40 μg per milliliter. Levels were somewhat higher and more prolonged when probenecid was administered concurrently. X-1497 left the serum rapidly when given either intramuscularly or intravenously, and 4 hour levels were scarcely in the therapeutic range with either mode of administration.

Twenty-three patients were treated with 4 to 6 Gm. of X-1497 daily. Included were 11 cases of staphylococcal infection, in 9 of which there was improvement. Two cases of streptococcal cellulitis and 7 cases of probable pneumococcal pneumonia were cured with 5 days of therapy. Side effects were negligible.

X-1497 shows promise of being a safe and effective antistaphylococcal agent.

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Addendum

Since this manuscript was prepared, a number of articles on dimethoxyphenyl penicillin have appeared in the British literature using the name "BRL 1241" or "sodium 6-(2,6-dimethoxybenzamido) penicillanate monohydrate." Some of them are listed below.

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Some effects of nicotine and smoking on metabolic functions

A number of the metabolic effects of nicotine (or tobacco smoke) reported in mammals are reviewed, and attention is drawn to the relative deficiency in this area of investigation. The suggestion is made that, concomitant with the advance of newer and more sophisticated biochemical and biophysical techniques, the effects of nicotine be more systematically studied using concentrations that include those encountered in man.

In general, nicotine appears capable of increasing heat production, oxygen consumption, metabolic rate, and blood sugar. Studies of tissue metabolism show that nicotine can inhibit pyruvate oxidation by inhibiting specifically pyruvic dehydrogenase. Nicotine has been shown to possess anticholinesterase activity and to suppress acetylcholine synthesis. These observations may all be of importance in finally elucidating the mechanism of the neurotropic action of nicotine and deserve to be extended.

The uncomplicated effect of nicotine on serum cholesterol and lipoprotein levels is not yet clearly apparent. Generally, the levels appear to be higher in smokers than in nonsmokers; the reason for the difference is obscure. Effects of nicotine on nitrogen metabolism are virtually unknown; effects on mineral metabolism, when demonstrable, appear indirect and to lack both consistency and significance. Vitamin metabolism is largely unaffected, except for ascorbic acid, which reportedly may be depleted to a perhaps significant extent.

It is interesting and significant to note that there are few clinical reports which implicate nicotine (or tobacco smoke) etiologically or otherwise in deficiency or metabolic disorders.

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There is a very great deal of published work, including innumerable reviews (some of which we ourselves have perpetrated), concerned with the not unimportant question of what, in the words of Comroe,[22] nicotine *can* do. Much of it gives the impression (no doubt quit unintentional on the part of the authors) that such pharmacodynamic actions belong, so to speak, to nicotine rather than to the organism acted upon or, more precisely, than to its ultimate structure. But, as Sollmann[153] so cogently states in the prelimi-

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naries to his classic text, "Pharmacologic agents rarely if ever create new functions in a cell or tissue; they only modify existing functions or make evident functions which have previously been latent." Thus, nicotine exerts its effects not, for example, on blood pressure, but rather on certain tissues, cells, or intracellular components (e.g., enzymes). These effects are sporadically demonstrable as experimental techniques are developed. This review—which we believe to be the first of its nature—is an agglomeration (the paucity of data really permits no more synthetic term or treatment) of observations on certain effects of nicotine or tobacco smoking on certain selected metabolic functions in the intact organism or in organs or tissues or cells or enzyme systems. For the most part, the observations dealt with here are concerned only with warm-blooded animals, and, whenever possible, relevant clinical implications with respect to smoking by man have been included. But if animal experimentation on the metabolic effects of nicotine has been relatively sparse, clinical investigations of the influence of smoking on diseases of metabolism seem to have been absolutely scanty. This latter phenomenon may indicate that normal smoking has no such influence—as appears to be the case with diseases of the muscular system (Fischer and colleagues[41])—or else that no one has searched for it. For medical science owes far more to *Matthew* 7:7 than to the *Three Princes of Serendip:* "Seek, and ye shall find" is a more productive working principle than serendipity.

Heat and energy exchange

Effect of nicotine on oxygen consumption. Although it would be hazardous to compare the effects of nicotine in the respiration of yeast cells to that of animal, particularly of mammalian, cells, one may take preliminary note that relatively low concentrations of nicotine reportedly accelerate yeast fermentation of sugar (Liebig[105]; Somogyi[154]). Larger concentrations decreased the fermentation rate,[154] as did exposure to tobacco smoke (Loeser[106]; Küchle and associates[97, 98]; Grosskinsky and Stürmer[53]). Apparently, the responsible constituent in the smoke was neither nicotine nor carbon monoxide nor cyanide.[53]

Cugurra and Baldini[23] determined the metabolic rate of young rats at room and at low temperatures, with and without nicotine; at 20° C., subcutaneous injection of 10 mg. nicotine bitartrate per kilogram (0.1 minimal lethal dose) was said to have had a distinct inhibiting effect on oxygen consumption, with more marked effects observable at temperatures of –8° and –10° C. Males[111] had previously reported experiments on two rats in which 3 to 10 mg. nicotine caused a very brief caloric increase, accompanied by muscular contractions and general excitation, followed by a more prolonged heat production somewhat below normal.

In anesthetized dogs, total body oxygen consumption was increased during infusion of 0.015 mg. nicotine per kilogram per minute (Schmitthenner and co-authors[150]).

In *chronic* experiments on guinea pigs injected with nicotine, oxygen consumption rose during the first 12 days, then fell away gradually to approach normal at about 40 days; finally, at 50 to 60 days, the oxygen consumption was slightly below normal (Erbacher, Grumbrecht, and Loeser[31]).

Basal metabolic rate of smokers. Schlumm[146] found an average increase in basal metabolic rate in "heavy smokers" of +20 per cent and an average respiratory quotient of 0.80, the latter being considered somewhat below normal for a mixed diet. In a later article,[147] he reported that 55 of 71 heavy smokers showed an elevated basal metabolic rate, averaging +21.7 per cent. There was said to be no correlation between the amount of tobacco consumed and the level of the basal metabolic rate; but in 8 heavy smokers tested, the increased metabolic rate paralleled an increased blood iodine concentration, and this relationship was thought to be significant (see below). When smoking was dis-

continued for a few days, the basal metabolic rate usually returned to normal, the respiratory quotient quite regularly rose, and the patient often began to gain weight.[146] (The subject of weight gain on stopping smoking is discussed below.) In contrast to Schlumm's findings, Silbert and Friedlander[152] reported that 12 male heavy smokers showed an average basal metabolic rate of –15.1 per cent. Two of these were repeatedly examined after they stopped the use of tobacco, but no striking change in metabolism occurred. In 50 patients with thromboangiitis obliterans, the average basal metabolic rate was –16.2 per cent; the average of those patients who had given up smoking was –16 per cent, that of those who were still smokers, –16.5 per cent.

In an unselected series of 1,200 consecutive patients, both men and women, Hadley[56, 57] reported that the average basal metabolic rate of 711 nonsmokers was –3 per cent and of 489 smokers was +0.9 per cent. This difference was said to be statistically significant, but such a statement must, of course, be taken in a mathematical rather than in any physiologic sense, as is also true of the more recent report by Brožek and his colleagues.[16] In 265 middle-aged American men (45 to 55 years) examined by these latter workers, basal pulse rate and oxygen consumption were found to be slightly higher in cigarette smokers (more than ten cigarettes daily) than in nonsmokers; the differences approached significance at the 5 per cent level of probability.

Effect of cigarette smoking on metabolic rate and oxygen consumption. In general, smoking one to two cigarettes has been found to have little effect on the metabolic rate or oxygen consumption in man (Dill, Edwards, and Forbes[28]; Dagnino[24]; Hackfield[55]; Hiestand, Ramsey, and Hale[67]; Goddard and Voss[49]; Evans and Stewart[33]; Stewart, Haskell, and Brown[157]; Juurup and Muido[83]; Henry and Fitzhenry[66]; Roth, McDonald, and Sheard[138, 139]; Roth[137]; Baroni and Mandrioli[7])—at least allowing for the generally accepted "normal" range of ±10 per cent and for the fact that the purely physical act of "smoking" cannot fail to have a *non*drug influence on basal metabolic rate or even on nonbasal metabolic rate. If the question of the influence of smoking on metabolic rate is to be satisfactorily answered, clinical experiments of more sophisticated design than those previously undertaken must be carried out; but it is perhaps a more fundamental question to ask whether an answer, in any case, would have any particular interest or value with respect to the understanding of smoking.

Smoking and thyroid activity in man. Several observers (Schlumm[146, 147]; Strauss and Scheer[161]; Gutzeit and Parade[54]; McIlvaine[119]) have pointed out certain resemblances between the signs and symptoms seen in heavy smokers or "chronic nicotine abuse" and in hyperthyroidism, e.g., tachycardia and palpitations and other heart complaints, increased blood pressure, moist skin or increased sweating, tremor, dizziness, nervous symptoms, shining eyes and excitement, loss of flesh. To Schlumm,[147] this indicated an effect of nicotine on the thyroid in the direction of an increased level of secretion, a conclusion also reached by Scheer.[144] Strauss,[160] indeed, claimed to have demonstrated increased thyroid hormone activity in blood taken from smokers given nicotine over several days to the point of nicotine poisoning. Blood iodine curves obtained were also held to be an indication of heightened thyroid activity (Schlumm[147]; Strauss and Scheer[161]) although analogous blood iodine curves were found for epinephrine by Schittenhelm and Eisler (cited by Strauss and Scheer[161]) and after exercise (Gutzeit and Parade[54]). Gutzeit and Parade, therefore, postulated that the rise in blood iodine observed by them might be the result of increased epinephrine, rather than of increased thyroid secretion.

The clinical picture of "nicotine-sensitive" and "nicotine-damaged" individuals described by Strauss and Scheer[161] was said

by these writers to be similar to that seen in many cases of hyperthyroidism, with which disease it was often confused, especially among women. Recently, Jackson[81] maintained that many cases diagnosed as hyperthyroidism were the result of nicotine or coffee abuse. Of 228 patients diagnosed elsewhere erroneously as having hyperthyroidism, 112 were said by Jackson to be actually suffering from "nervous tension exhaustion," mainly because of overindulgence in the use of caffeine (coffee, tea) and nicotine. Directly or indirectly, these "stimulants" were responsible for nervous tension, palpitation, tremor, weight loss, and insomnia, and their use was so important in the condition of these patients, and occurred so often, that the terms "nicotinitis" and "coffeeitis" were coined by the author to express this trouble. Patients who used these stimulants excessively presented a picture of pseudohyperthyroidism.

Other writers have gone even further, however, and stated that smoking can cause real, rather than pseudo, thyroid dysfunction (Schmidt[148]; Szentiványi, Földes, and Veress[164]), especially under conditions of deficient iodine intake, as in the "goiter belt" (McIlvaine[119]). Bernhard[9] compared the clinical records of 458 women who smoked regularly (at least three cigarettes daily) with those of 5,000 who did not smoke but who were said to be otherwise comparable to the smokers. Of the smokers, 33.1 per cent were said to be hyperthyroid and 6.8 per cent hypothyroid, compared to 5.4 per cent and 0.4 per cent, respectively, of the nonsmokers.

Smoking and body weight. Several of the above-mentioned writers have equated the weight loss in "chronic nicotine abuse" with increased thyroid function. By the same token, the weight gain so often noted in persons who have stopped smoking should be due to decreased function of this endocrine gland. Most observers, however, have offered a more simple—and more credible—explanation of this latter phenomenon: people who stop smoking start overeating, or, at least, eating more. Everyday observation of "chainsmokers" (who would probably be considered by some of the above authors to be suffering from chronic nicotine abuse) leads one to suspect that the reason they have "lost weight" is more apt to be that they eat less than their nonsmoker or exsmoker contemporaries. Thus, weight loss in excessive smokers and weight gain in exsmokers would appear to be due to an imbalance, in each instance, between caloric intake and expenditure—in other words, a metabolic rather than an endocrine matter.

Effect of nicotine and smoking on body temperature. It has been reported that nicotine caused an increase in body temperature in the rabbit (Falck[38]; Hogyes[71]; Döblin and Fleischmann[29]; De Gaetani[26]), while ear temperature fell (Kanitz and Sellschopp[84]), the mechanism here being quite obviously peripheral vasoconstriction with decreased heat loss. Other writers have reported a fall in body temperature after the administration of nicotine to guinea pigs (Leschtschinskaja[104]), rabbits (Tscheschichin[176]; Tamassia[166]; Leschtschinskaja[104]; Tiba[169]), dogs (Tamassia[166]; Franke and Thomas[44]), and even a cow (Hatcher and Crosby[62]). According to d'Arzignano,[2] body temperature was usually somewhat elevated by small doses of nicotine and fell after lethal doses, especially in the period of paralysis; the total variation from normal was usually only 0.5° to 2° C. It is, of course, to be expected that death occurring during or soon after convulsions from any cause would be characterized by an elevated body temperature, whereas any prolonged period of paralysis, from equally nonspecific causes, would result in a fall. Thus, Franke and Thomas[44] reported a constant tendency toward a fall in body temperature of nicotine-poisoned dogs during the paralytic state; but other authors have not furnished this information, without which it is impossible to interpret their results.

Maren[112] demonstrated an antipyretic effect of nicotine on fever produced by yeast injection in rats, rabbits, and dogs.

He concluded that nicotine was sixty times as active as salicylic acid and ten times as active as the most potent cinchoninic acid derivatives. The reader should not forget, however, that nicotine is many times as toxic as well.

Two older reports state that body temperature is very slightly elevated in smokers (Troitski[175]; Lachowiecki[99]), and, no doubt, the thermometer readings obtained were more accurate than the conclusion derived therefrom. Several workers have since tested the effect of cigarette smoking on oral and/or rectal temperature. Maddock and Coller[110] reported that normal subjects smoking in their usual manner showed no significant changes in mouth temperature, although skin temperature generally decreased. Rectal temperatures rose as a result of smoking two-thirds of two cigarettes (Evans and Stewart[32, 33]; Stewart, Haskell, and Brown[157]). According to Roth, McDonald, and Sheard,[138, 139] the maximum rise of oral temperatures after smoking two standard cigarettes was 0.6° C., but there was little change in rectal temperature. Since thermometry is, or ought to be, quite accurate, these discordant results are probably a reflection of differing experimental conditions. In any event, they would appear in general to be merely reflections of the peripheral vasoconstriction commonly caused by smoking.

A note in the *Journal of the American Medical Association* (165:424, 1957) stated that cigarette and pipe smoking tests were carried out on 5 habitual smokers, the oral temperature being obtained before smoking and again 1 minute and 5 minutes after the completion of the smoking of a cigarette and after 10 minutes of continuous pipe smoking. The greatest rise obtained was 0.1° F. 1 minute after smoking.

Carbohydrate metabolism

Effect of nicotine and tobacco smoke on blood sugar in animals. Virtually all investigators agree that injection of nicotine in animals results in a measurable, and often a marked, increase in blood sugar (dog: Underhill[178]; Houssay and Molinelli[74, 75]; Burstein and Goldenberg[17]; Hazard and Vaille[63]; Wada[193]; Watanabe[196]; Kojima, Endo, and Nagakura[94]) (rabbit: Imahasi[76, 77]; Nicolaysen[129]; Inaba and Oikawa[79]; Ssalischtscheff[155]; Töppner[173]; Hiraoka[69]; Lee[101, 102]; Wada[193]; Watanabe[195]; Kobayashi[91, 92]; Tiba[170]; Van den Heuvel-Heymans[179]) (guinea pig: Mosinger and associates[125]) (rat: Wilson and DeEds[205]). In general, rises in blood sugar after subcutaneous injection of nicotine were proportional to the dose used (Nicolaysen[129]; Hazard and Vaille[63]; Kobayashi[92]).

Houssay and Molinelli[75] reported that adrenalectomized dogs showed the same rise in blood sugar after nicotine as did normal dogs; other workers, however, have reported that removal or demedullation of the adrenals in the dog greatly diminishes or abolishes the hyperglycemic response to injected nicotine (e.g., Leloir[103]; Hazard and Vaille[63]; Wada[193]; Kojima, Endo, and Nagakura[94]). In the rabbit also, double adrenalectomy greatly lessened the effect of nicotine on blood sugar (Inaba and Oikawa[79]; Kobayashi[92]; Tiba[170]; Van den Heuvel-Heymans[179]). For the most part, section of the splanchnic nerve in dogs had no effect on their subsequent blood sugar response to injected nicotine (Leloir[103]; Watanabe[196]; Wada, Hirano, and Tiba[194]). However, Leloir did find a diminished hyperglycemic response to nicotine for a short period (3 to 5 hours) after splanchnic section which he attributed to the effects of anesthesia and the surgical manipulation. In 7 days, the responses in the splanchnicotomized dogs were essentially the same as in the controls.

In rabbits, splanchnicotomy, whether relatively acute or chronic, according to Inaba and Oikawa,[79] Kobayashi,[92] and Tiba[170] weakened only slightly the hyperglycemic effect of nicotine. On the other hand, Watanabe[195] found that after section of the splanchnic nerves in rabbits, the resulting nicotine-induced hyperglycemia was strongly inhibited.

A number of investigators have tested the effect of sympatholytic drugs on the hyperglycemic response to nicotine. The nicotine hyperglycemic effect was found to be depressed by ergotamine (rabbit: Imahasi[77]; man: Caponnetto[18]; Andrea[1]), by yohimbine (rabbit: Imahasi[76-78]), or by sparteine (dog: Hazard and Vaille[63]). However, in the dog, Leloir[103] found that ergotamine did not materially alter the blood sugar response to nicotine.

Since nicotine hyperglycemia is reduced or abolished in animals deprived of their adrenal medullae but not in splanchnicotomized animals, the blood sugar rise after nicotine injection must be attributed in large part to a direct stimulation of the adrenal medulla (Burstein and Goldenberg[17]; Leloir[103]; Scheer[144]; among others). Confirmatory evidence is furnished by cross-circulation experiments in which the adrenal vein of a donor dog is anastomosed to the jugular of a recipient animal. In such preparations, nicotine injection into the donor results in a marked rise in blood sugar in the recipient (Houssay and Molinelli[74, 75]; Leloir[103]). The effect of the sympathicolytic drugs in inhibiting nicotine hyperglycemia (described above) is also suggestive.

That other, extra-adrenal factors are operative as well, however, is indicated by the fact that adrenalectomy or adrenal demedullation does not entirely suppress the blood sugar rise that normally results from nicotine injection. Extra-adrenal factors which have been suggested are: sympathetic tonus (Ssalischtscheff[155]), epinephrine secretion by extra-adrenal chromaffin system or sympathin secretion by the sympathetic nerves (Leloir[103]), stimulation of the entire sympathicoadrenal system (Short and Johnson[151]), and central or ganglionic stimulation leading to increased adrenal secretion (Van den Heuvel-Heymans[179]). Recent work suggests the additional possibility that small doses of nicotine may excite the chromaffin cells of the adrenal medulla reflexly via the carotid sinus and other chemoreceptors. That one of the extra-adrenal factors is not reduced pancreatic secretion is indicated from certain experiments of Foglia,[43] who found that when the pancreas was grafted into the neck of a dog, perfusion of the gland through its arterial supply with nicotine had no effect on the blood sugar level.

Effect of smoking and nicotine on blood sugar in man. In contrast to the near unanimity of results after the injection of controlled (and relatively large) doses of nicotine into animals, the effect of smoking on the blood sugar levels in man is notably inconsistent. Some writers have reported more or less hyperglycemia (Andrea[1]; Caponnetto[18]; Lundberg and Thyselius-Lundberg[107]; Töppner[173]; Boldyreff[13]; Short and Johnson[151]; Drucquer[30]; among others); other workers have found and reported hypoglycemia (e.g., Ssalischtscheff[155]; Sakoschansky[142]); still others, no change Burstein and Goldenberg[17]; Cates and Giovanaszzi[21]; Dagnino[24]; among others); while other series of subjects have included those who reacted to smoking in any one of these three ways (Scheer[145]; Högler[70]; Garcia[46]; Wachholder[192]; among others). When rises in blood sugar are reported, they are generally less marked than those observed after nicotine injection into animals; but this is undoubtedly merely a reflection of relative dosage. Indeed, many, if not most, of the discrepancies recorded in man are very probably due to differences in effective dosage of nicotine absorbed by the several subjects, and recent "standardized" smoking tests have given fairly uniform results. For example, Rehder and Roth,[135] testing 24 normal subjects under basal conditions, found that smoking two-thirds of two cigarettes resulted in no appreciable rise in the levels of the fasting blood sugar and in epinephrine-like substances of the systemic venous blood. These authors also reported a personal communication from D. S. Cristol, who was unable to demonstrate any change in the level of the fasting blood sugar in 175 medical students after they smoked three-fourths of two cigarettes. Also, repeated observations

by Berry[10] failed to show a consistent increase in blood sugar after the smoking of two cigarettes in rapid succession.

Haggard and Greenberg[58] found that when the respiratory quotient was above 0.85 and the blood sugar correspondingly above 130 mg. per 100 ml., the smoking of a cigarette had no appreciable influence on either; but when the quotient and the blood sugar had fallen below these values and especially when the fasting level had been reached, the smoking of a cigarette was followed by a rise in both. These authors' contention was challenged by Dill, Edwards, and Forbes[28]; although there is something inherently probable in the concept (which Wilder[203] has termed the "law of initial value") that the response of a drug is dependent upon the state of the relevant function or end organ at the time of stimulus. In fact, Wachholder[192] found the effect of cigarette smoking on blood sugar to be dependent upon its initial level: when low, a small rise occurred; when high, a small drop. Perhaps a similar "biphasic" response is that described by von Kreuziger and his co-workers.[191] According to these investigators, two different reaction patterns were observed on smoking one cigarette in about 10 minutes: (1) immediate neutrophilia with a relative lymphopenia and initial blood sugar decrease and (2) initial lymphocytosis with a relative neutropenia and immediate blood sugar increase. These changes coincided with the strongest subjective discomforts and were attributed to epinephrine release.

Wachholder[192] investigated the comparative results on fasting blood sugar of pipe, cigar, and cigarette smoking in 5 nonsmokers and 14 habitual smokers, all young men. In habitual smokers pipe smoking tobacco containing about 0.5 per cent nicotine, blood sugar rose 20 to 40 mg. per 100 ml., the rise beginning in the first minute; in some subjects the peak occurred during smoking, in others after finishing; return to normal occurred 30 to 60 minutes after smoking was completed. With stronger (0.94 per cent nicotine) pipe tobacco, the blood sugar rise still occurred, but was of smaller magnitude and often returned to normal during smoking, or lasted at most 30 minutes after smoking before falling below normal. With nicotine-free tobacco, blood sugar rose as strongly as with tobacco that contained 0.5 per cent nicotine, but the rise was of shorter duration and at best lasted only a little beyond the smoking period, with subsequent drop below normal levels. Smoking mild cigars (about 1.1 per cent nicotine) caused a 50 to 70 mg. per 100 ml. rise in blood sugar in habitual smokers; the rise was of shorter duration than that observed with pipe tobacco, usually fell to about normal during smoking of the second half of the cigar, and dipped slightly below normal after smoking. With strong (1.7 per cent nicotine) cigars, the blood sugar rise was less and was often followed by a drop to well below normal. In experiments in which nonsmokers and habitual smokers participated, smoking two cigarettes in succession without inhalation of the smoke caused a smaller and more fleeting blood sugar rise than that observed with pipe and cigar smoking; in one-third of the subjects, the rise remained within the experimental error. With "strong" cigarettes, blood sugar levels often dropped below control during smoking or thereafter. Virtually nicotine-free cigarettes (0.09 per cent nicotine) caused little or no blood sugar change. In this connection, it may be noted that intramuscular injection of 1 mg. nicotine or nicotine hydrochloride resulted in considerable blood sugar increases (Caponnetto[18]; Andrea[1]), although intravenous injections of 2 mg. nicotine bitartrate were said to have had no consistent effect on blood sugar (Boyle and coauthors[15]). It would appear, then, that the ingredient in tobacco smoke primarily responsible for its hyperglycemic effect is most likely nicotine; and, it may be noted, dogs made to inhale cigarette smoke generally showed hyperglycemic responses similar to those observed after nicotine injection (Tournade

and Malmejac[174]; Boldyreff[13]; von Kreuziger, Kemper, and Heinecker[191]). A case of accidental nicotine poisoning reported by von Ahn[189] obviously illustrates the pure nicotine effect. In the patient, the blood sugar about 14 hours after poisoning was 218 mg. per 100 ml.; the next day, 170 mg. per 100 ml.; and on the third day, 108 mg. per 100 ml. These levels approximate those found in animals after comparable toxic dosage.

Effect of chronic nicotine administration on blood sugar in animals. Chronic administration of nicotine was said to have resulted in hyperglycemia (guinea pig: Mosinger and co-workers[125]; rabbit: Matsuoka[115]), hyperglycemia progressively lessening to become an ultimate hypoglycemia (rabbit: Kobayashi[91, 92]), or no significant changes in blood sugar levels (rat: Wilson and DeEds[205]; Erbacher, Grumbrecht, and Loeser[31]; dog: Burstein and Goldenberg[17]). In general, those doses of nicotine which did not give rise to gross poisoning were without effect, whereas frankly toxic doses resulted not only in hyperglycemia but also in nonspecific nutritional disturbances. The classic dilemma of such "chronic" experiments is thus again apparent: if the dosage of nicotine is small enough to approximate that obtaining in human smoking, no effects are observable; if each "chronic" dose results in acute poisoning, the long-term results may be merely a result of repetitive acute poisonings, which, it is reasonable to suppose, do not add up to "chronic" poisoning.

Effect of habitual smoking on blood sugar in man. Latzel[100] reported that the average blood sugar of 5 habitual smokers during a period of smoking regular cigars and cigarettes was 80 mg. per 100 ml. During a period of smoking 40 per cent denicotinized cigars and cigarettes, the blood sugar averaged 117 mg. per 100 ml.; during periods of abstinence from smoking, 103 mg. per 100 ml. The author explained these blood sugar changes as follows: nicotine caused increased gastric secretion, which was an indication of vagus stimulation; vagus stimulation caused secretion of insulin, which lowered the blood sugar during the smoking period. During decreased nicotine intake, the reverse changes occurred.

A condition termed "tobacco hypoglycemia" was described in a preliminary report by Bohan,[11] and two case histories were given in detail. Later, Bohan and Berry[12] reported that 36 of 38 patients in whom hypoglycemia was said to be the only significant abnormal finding were excessive users of tobacco and that those patients who discontinued tobacco use were free of symptoms ascribed to hypoglycemia within a month, while some were dramatically relieved when they stopped smoking. In the few patients who resumed smoking, hypoglycemic symptoms recurred after several months but again subsided when the use of tobacco was stopped a second time. In a few patients who had smoked 30 to 40 cigarettes daily, symptoms subsided when they reduced their consumption to just over 10 a day. According to Berry,[10] the diagnosis of tobacco hypoglycemia was made only in those patients who met all of the following criteria: (1) the use of one or more packages of cigarettes (or equivalent) a day, (2) symptoms compatible with the diagnosis of hypoglycemia, (3) venous blood sugar (Folin and Wu method) below 55 mg. per 100 ml. concurrent with the symptoms, (4) prompt relief by the ingestion or administration of glucose at the time of occurrence of symptoms, (5) complete and permanent relief of symptoms upon cessation of smoking. Case histories of 7 such patients were described. Although the mechanism by which hypoglycemia can be produced by tobacco was unknown to Berry (presumably, Latzel's explanation—above—would provide a mechanism, however improbable), he stated that tobacco hypoglycemia is a clinical entity which should be considered in the differential diagnosis of patients with evidence suggestive of either islet cell or "functional" hypoglycemia. Bohan and Berry[12] suggested that tobacco hypoglycemia be ruled

out before any patient is subjected to complete or even partial pancreatectomy.

Regarding diabetes mellitus, little or no statistical association was evident in the well-known prospective study of Hammond and Horn[59] between cigarette smoking and this disease. However, Vincent, Segonzac, and Lagreu[186] stressed the ill-effect which would result in diabetes from repeated discharge of epinephrine (resulting in hyperglycemia) in smoking. (Diabetics, incidentally, also show blood sugar increases on smoking or nicotine administration—Andrea[1]; Töppner[173]; Lundberg and Thyselius-Lundberg[107].) On the basis of their studies described above, Rehder and Roth[135] concluded that elevation of the blood sugar related to cigarette smoking is not a likely cause of erroneous diagnosis of diabetes mellitus.

Effect of nicotine on glycosuria. Töppner[173] credited Bucelli (1896) and von Stern[156] with having first observed sugar in the urine as a result of nicotine poisoning. Von Stern also observed that the habitual or excessive use of tobacco could aggravate a glycosuric condition, whether alimentary or diabetic in nature; he presented protocols of several cases of diabetes in which smoking increased the glucose excretion and also 1 case diagnosed as diabetes by several physicians, in which reduction in smoking led to complete disappearance of the glycosuria without imposition of any diet. Since glucose was often found in the urine in chronic carbon monoxide poisoning, von Stern believed this to be the causative factor in glycosuria of tobacco smoke origin.

In animals rendered glycosuric by puncture of the medulla or otherwise, sufficiently large doses of nicotine inhibited the glycosuria (see Macleod and Dolley[109]; King[88]), but the mechanism of this complicated and obscure effect is apparently related to the ganglioplegic effects of such doses of nicotine and not to "metabolism" in the sense of this review.

Effect of nicotine on glycogen. In rabbits, large doses of nicotine lowered the liver glycogen content (Kobayashi[92])—a not unexpected result in the light of the simultaneous blood sugar increase. For effects on muscle glycogen, see below under organ and tissue metabolism.

Effect of nicotine on lactic acid. In rabbits given subcutaneous injections of nicotine, blood lactic acid levels increased, and this increase was only slightly inhibited after section of the splanchnic nerves (Watanabe[195]). In man, however, smoking one cigarette with inhalation had no appreciable effect on the lactic acid concentration in venous blood (Dill, Edwards, and Forbes[28]; Freund[45]).

In mice thrown into convulsions by nicotine, brain lactic acid values did not differ significantly from those obtained on normal animals (Stone[159]). Also see below, under organ and tissue metabolism: muscle.

Fat metabolism

Effect of nicotine on blood cholesterol in animals. According to Strauss and Scheer,[161] rabbits or dogs given single or repeated injections of nicotine had blood cholesterol curves which generally rose or fell inversely to the blood iodine values. Maslova[113] reported that the cholesterol content of the blood showed more marked increases in chinchilla rabbits given 2 mg. nicotine plus 0.2 Gm. cholesterol daily for several months than in rabbits that received only cholesterol over the same period of time. Using New Zealand white rabbits, Wenzel and Beckloff[198] studied twelve animals on a control diet, a second group on a diet containing 0.1 per cent cholesterol, a third on 2.28 mg. nicotine per kilogram per day in the drinking water, and a fourth on combined cholesterol and nicotine. At the end of 28 weeks, the administration of nicotine in addition to a cholesterol-containing diet had caused significant increases in plasma cholesterol, lipid phosphorus, and the cholesterol/phosphorus ratio. The authors stated that the importance of the nicotine enhancement of hypercholesterolemia as an atherogenic stimulus may be negated by the concomitant rise in lipid

phosphorus, as reflected by the cholesterol/phosphorus ratios. In a later communication, Wenzel, Turner, and Kissil[200] reported that serum cholesterol and phospholipid values of rabbits fed cholesterol or cholesterol plus nicotine over a period of 24 weeks were not affected by the addition of nicotine; but in these latter experiments, the dose of cholesterol used was 1 per cent, and it was thought that this higher dosage may have obscured any nicotine effect.

In cockerels given 1 per cent cholesterol in their diet and 2.2 mg. nicotine per kilogram daily in drinking water for 16 weeks, the plasma cholesterol and lipid phosphorus levels and the pathologic cardiovascular condition were not significantly different from those of the controls on cholesterol (Wenzel, Turner, and Kissil[199]).

Effect of smoking on blood cholesterol and lipoprotein. Blood cholesterol curves obtained by Strauss and Scheer[161] in persons who smoked eight to twenty cigarettes were said to have been of two types: one correlated with occasional smokers, nicotine-sensitive individuals, and hyperthyroid patients, in whom smoking caused an increase in blood iodine, and a second type correlated with habitual smokers and hypothyroid patients, in whom smoking caused a decrease in blood iodine. Short and Johnson[151] reported that total blood cholesterol in 5 habitual smokers rose slightly after cigarette smoking; in a study of 15 persons, Högler[70] stated that blood cholesterol (total cholesterol and its ester) was not clearly influenced by smoking. Recently, Page, Lewis, and Moinuddin[132] found that the rapid smoking of two nonfilter tip cigarettes by habitual smokers or nonsmokers was without effect in modifying serum cholesterol or lipoprotein concentrations during a 30 minute period immediately after smoking, from which they concluded that if this form of smoking is a stress, it is insufficient to change blood cholesterol or lipoprotein levels during short periods of time.

Kerschbaum and associates[86a] studied serum free fatty acid levels in response to smoking two cigarettes. In all but 1 of 17 subjects, the levels rose at the end of the 10 minute smoking period; the maximum rise (98 to 590 μEq per liter, mean 292) usually occurred 10 minutes later, and, in most instances, free fatty acids were still above the presmoking level 20 to 40 minutes after smoking. However, there was essentially no change in serum total cholesterol and triglycerides, and the authors concluded that cigarette smoking is followed by a mobilization of free fatty acid, probably effected by the stimulating action of nicotine on the adrenal glands and sympathetic nervous system, resulting in the release of free fatty acids from adipose tissue.

In connection with the current interest in atherosclerosis, a number of workers have studied, or are studying, cholesterol levels in populations of smokers and nonsmokers; but it should be pointed out that guessing the biologic significance of such statistical studies, however unexceptionably carried out, is always an unwarranted extrapolation. The authors' use of the word "significant" in the following reports must therefore be taken to apply solely to their mathematical procedures, only by coincidence to any conclusions.

Gofman and co-workers[50] analyzed for serum lipoprotein and cholesterol in several hundred healthy men and women divided into age and sex groups and, within these groups, by smoking habits. They reported that regular cigarette smoking was associated with an appreciable elevation of serum lipoprotein and cholesterol, particularly in young men of 20 to 29 years of age, the increase in this group being 21 per cent over the nonsmokers as compared to a corresponding 10 per cent increase in the group of 30 to 39 years of age. A different correlation with age was evident in an investigation of 189 healthy Boston men, aged 20 to 50 years, of Neapolitan ancestry (Miller and associates[122]). Those who smoked one pack or more of cigarettes per day had an appreciably higher mean serum total cholesterol level than did the

nonsmokers, and the differences were more marked as the age increased. However, in all age groups, the mean serum total cholesterol level was lower in those who smoked more than one pack of cigarettes than in those smoking only one pack.

In a study still underway in Framingham, Massachusetts, serum cholesterol levels tended to be slightly higher among cigarette smokers than among nonsmokers and higher among those who smoked and stopped than among those who had never smoked (Dawber and colleagues[25]). Among Johns Hopkins medical students, Thomas[167] found smokers to be in the minority at low serum cholesterol levels, whereas at higher levels, the smokers predominated. The data do not indicate, however, whether smoking increases the level of cholesterol or whether students with higher cholesterol levels are more likely to smoke. A subsequent experiment by Thomas and Eisenberg[168] designed to study the effect stopping smoking has on cholesterol levels was said to be inconclusive because of unforeseen difficulties. The volunteers to stop smoking showed an interesting degree of self-selection in the direction of very low cholesterol values, compared to their classmates; the 6 volunteers, with low cholesterol values to begin with, showed no definite tendency to develop significantly lower cholesterol values during the "stop smoking" experiment (which was continued for less than 2 months).

In healthy men aged 20 to 59 years in rural areas of West and East Finland and in Helsinki, serum cholesterol was found to be significantly higher in those who smoked cigarettes daily than in men who had never smoked (Orma and coauthors[131a]; Keys, Karvonen, and Fidanza[86b]; Karvonen and associates[84a]).

In men who manifested an intense, sustained drive for achievement and were continually involved in competition and deadlines, both at work and in their avocations, serum cholesterol level (and incidence of coronary artery disease) was found by Friedman and Rosenman[45a] to be much higher than in groups that manifested the opposite sort of behavior pattern; but this difference did not appear to be related to the smoking habits. Similar findings, and a similar conclusion with respect to smoking, were also reported for females (Rosenman and Friedman[136a]).

Perhaps it should be emphasized that in most of these recent studies of what has been called the "coronary question," the factor of smoking is but one of several or many factors studied (for example, Rosenman and Friedman recorded "history, habitus, blood cholesterol, clotting and lipoproteins, electrocardiogram and diet, exercise, smoking and other habits"), so that, in necessarily staying within bounds of a "smoking" review, we may seem to be narrowing the scope of what are, in many instances, admirably wide-ranging studies.

In making chylomicron determinations, Moreton[124] stated, the blood specimen before a breakfast meal containing fat should be taken with the subject refraining from drinking water or smoking, because "drinking water or smoking may wash out or squeeze out, by the first active peristalsis of the morning a few chylomicrons which may in an occasional individual remain in the lacteals as a result of fat ingestion of the day before. This would result in the erroneous impression of fasting chylomicronemia of internal, metabolic (pathologic) origin." Becker, Meyer, and Necheles[7a] made blood chylomicron curves on 30 normal healthy individuals aged 10 to 34 years before and after a fat meal and reported that 5 subjects showed prolonged and elevated curves. One of these latter was a young adult who had smoked a number of cigarettes during the test. Following up this observation, Marder and colleagues[111a] studied a group of 6 young (20 to 30 years) and 3 older (68 to 83 years) subjects who smoked one standard cigarette each during the 5 hour experimental period after a standard fat meal. As compared to results during the nonsmoking period after a fat meal, 4 showed a marked increase and prolongation of both chylomicron and nephelo-

metric curves. In another individual who did not normally smoke, there was an even more pronounced effect. Tests were also made on 1 old and 1 young individual with smoking after ingestion of a fat-free meal, and no rises were found for the 5 hour period. Several possible mechanisms were suggested to explain this response to smoking: (1) increased heart rate and blood pressure might bring about circulatory changes which might cause the change, (2) decreased gastric motility and increased intestinal motility might play a role, (3) active peristalsis caused by smoking might "push" out chylomicrons from the lacteals, or (4) gastric acidity might be decreased.

Nitrogen metabolism

Ito[80] determined the urinary constituents of male rabbits and reported that nicotine inhibited the intermediary decomposition of albumin. Protein metabolism and its intermediary metabolism were also said to have been diminished after administration of nicotine to dogs (Tokizaki[172]).

In well-controlled experiments, Heller[65] demonstrated that rats fed 0.5 mg. nicotine daily in their diets (stated to correspond to a 10 mg. intake of nicotine in man) showed slow but definite increases in urinary carbon/nitrogen and oxygen/nitrogen quotients during the periods of nicotine feeding. In all cases, the basis for the increase was a decrease in the urinary nitrogen value. Heller concluded that this amount of nicotine caused a definite change in metabolism in the direction of inhibition of oxidative capacity. During the tests, the urine remained free of albumin as well as sugar or other reducing substances.

Effect of smoking on nitrogen metabolism in man. Lachowiecki[99] stated that nitrogen metabolism in nonsmokers was slowed by the smoking of seven to fifteen cigarettes daily and that assimilation of nitrogen-containing material from food was favored. Use of twenty cigarettes daily increased nitrogen metabolism, and nitrogen assimilation was said to be either unaffected or slightly disturbed. In experiments on themselves and 2 others, Gramatczikow and Ossendowsky[52] determined nitrogen in food, in urine, and in feces; they reported that smoking, especially in nonsmokers, disturbed the relation of urinary nitrogen to assimilated nitrogen, the intensity of the metabolic disturbance varying with the number of cigarettes smoked.

Latzel,[100] who studied 5 habitual smokers, reported that blood nonprotein nitrogen averaged 37 mg. per 100 ml. during periods of smoking regular cigarettes and cigars, 33.6 mg. per 100 ml. during smoking of 40 per cent denicotinized cigarettes and cigars, and 30.2 mg. per 100 ml. during abstinence periods; this downward trend was held to be significant. In a case of accidental nicotine poisoning, von Ahn[189] reported that the nonprotein nitrogen on the day of admission was 38 mg. per 100 ml.

Tsuru and co-workers[177] reported that total urinary nitrogen of smokers averaged 9.2031 Gm. per day, while that of nonsmokers was 8.5881 Gm. Knack[90] and Willis[204] both reported albuminuria in cases of acute nicotine poisoning. In one of Mattei's[116] cases, no albumin was found in the urine; in another, 4 per cent was detected on the first day of poisoning.

Uric acid metabolism. According to Högler,[70] after the smoking of three cigarettes in succession by fasting subjects, blood uric acid often rose at the height of the nicotine effect, although there had been no purine in the blood before the test [*sic*]. This effect, as many of the other metabolic effects, e.g., changes in blood calcium, potassium, and iodine (see below), could be secondary to nicotine stimulation of the adrenal medulla.

Mineral metabolism

Sodium; potassium; calcium. At the end of 3 weeks of daily subcutaneous injection of nicotine, the serum potassium level of rabbits rose about 5 mg. per 100 ml., and the serum calcium level about 4 mg. per 100 ml. (Scheer[143]). After an additional 6 day period of daily intravenous injections,

these rises were only slightly greater than before. Three days after cessation of nicotine administration, the levels began to fall and were approximately normal at the end of 3 weeks without nicotine. In acute experiments on rabbits and dogs, Strauss and Scheer[163] found that slow intravenous injection of 0.5 to 1 mg. nicotine tartrate per kilogram caused symptoms of intoxication and in most cases a more or less rapid elevation in blood calcium, which reached a peak in 15 to 30 minutes before returning to normal. Simultaneous administration of nicotine plus calcium led to a higher blood calcium level than did administration of calcium alone. Upon repeated nicotine injections in dogs, the second injection was found to cause a greater rise in blood calcium than the first, while the third injection resulted in a much smaller rise. In 4 out of 5 cases in adrenalectomized rabbits, blood calcium showed a slow, prolonged fall after nicotine administration. Under aprobarbital* narcosis, nicotine caused a rise in both calcium and potassium; the rises were prolonged and still present, though in reduced degree, 6 hours later. In a few experiments in which calcium, potassium, and inorganic phosphorus were studied, parallel rises in calcium and potassium occurred; phosphorus first fell, then rose to above control levels. In contrast to Strauss and Scheer's observation that under aprobarbital narcosis, nicotine caused a rise in blood calcium, Perrando[133] found in nicotine-poisoned rabbits the calcium level uniformly dropped when the animals were etherized, whereas in control rabbits the effect was more variable. With respect to potassium, Hazard and coworkers[64] found, in agreement with Strauss and Scheer, that injection of 0.1 mg. nicotine into dogs under chloralose and artificial respiration caused in 3 to 5 minutes a marked (60 to 80 per cent) increase in plasma potassium. After pretreatment with procaine or sparteine, nicotine caused little or no change in plasma potassium.

*Numal.

Inhalation of cigarette smoke by rabbits resulted in a fall in blood calcium, in which carbon monoxide and mild dyspnea were said to have played a role (Strauss and Scheer[163]). In dogs made to inhale the smoke from one cigarette, no change in blood calcium or sodium was detectable; blood potassium initially fell, then increased toward, but not to, control values within 30 minutes of the start of smoke exposure (von Kreuziger, Kemper, and Heinecker[191]).

Scheer[143] and Strauss and Scheer[163] studied the effect of smoking two to three cigarettes on the fasting serum calcium and potassium levels in nonsmokers and moderate and heavy smokers, reporting that the most frequent result of smoking was a rise in serum calcium from an average of 11.8 mg. per 100 ml. to as much as 16.8 mg. per 100 ml., the usual rise being of the order of 1 to 4 mg.. per 100 ml. The peak of the rise occurred on the average 15 minutes after the peak of intoxication symptoms (dizziness, salivation, perspiration, ill feeling), and the effect subsided in about 90 minutes. At the same time, the potassium level usually fell, the time of onset varying from the time of appearance of intoxication symptoms to 45 minutes. These results, which occurred in 70 per cent of the cases, produced a decrease in potassium/calcium quotient. In 9 per cent of the cases, the potassium increased and calcium decreased, resulting in an increased potassium/calcium quotient that lasted 45 to 60 minutes. No difference in reaction between young and grown individuals, and between smokers and nonsmokers, was discernible. Scheer considered that the fall in the potassium/calcium quotient was an expression of a sympathetic (sympathotonic) reaction to nicotine, while a rise in the quotient expressed a vagotonic reaction. Smoking but part of one cigarette in 5 to 10 minutes only occasionally caused a slight rise or fall in blood calcium. In a few experiments, Strauss and Scheer determined ionized as well as total calcium: ionized calcium rose with the rise in total

calcium and continued to rise at the point where the total calcium had begun to fall. In 2 subjects, smoking plus calcium administration caused a greater and more prolonged rise in blood calcium than did calcium administration alone; in another case, the calcium rise was less and fell off more rapidly. In contrast to these results, Högler[70] could find an important difference neither between the fasting calcium and potassium blood values, nor in the potassium/calcium ratio between smokers and nonsmokers, after 3 cigarettes were smoked in succession by fasting subjects. Von Kreuziger, Kemper, and Heinecker[191] found no change in blood calcium or sodium in subjects who inhaled the smoke from one cigarette. With respect to potassium, there were two types of change: (1) potassium initially decreased, then increased toward normal after 30 minutes; (2) potassium immediately increased on smoking and increased further after smoking. These findings were attributed to epinephrine release provoked by nicotine.

Von Ahn[189] noted hypokalemia (serum potassium 10.6 mg. per 100 ml.) in a case of nonfatal acute nicotine poisoning. Since this might have the effect of flattening the T wave of the electrocardiogram (a phenomenon sometimes noted after smoking), von Ahn[190] determined serum potassium in 7 subjects immediately before and after intravenous injection of 2 to 3 mg. nicotine, but no significant change was noted.

Phosphorus. Since atropine antagonizes nicotine contracture of striated muscle, Stewen[158] undertook to determine the effect of these drugs on intermediary phosphate metabolism. Using tissue breis of skeletal muscle, liver, or kidney of rabbits or cattle, he studied the effect of nicotine and atropine separately and in combination on the formation and degradation of hexosediphosphate and of glycerophosphate. Both atropine and nicotine were said to inhibit the formation and degradation of organic phosphates in tissue. Their combined action did not increase this effect, but sometimes decreased it; in only a few instances, in particular in the decomposition of glycerophosphate by liver tissue, was an antagonism (neutralization to the effect of nicotine by atropine) demonstrable.

Also see below under organ and tissue metabolism: muscle.

Iodine. Pursuant to the observation that the clinical picture of nicotine poisoning presents several common features with hyperthyroidism (see above), several investigators have studied the effect of smoking on blood iodine. Schlumm[147] found the blood iodine of 8 heavy smokers to average almost three times that of normal individuals; the basal metabolic rate was also increased, and the parallelism was considered significant. Schlumm's patients were apparently selected primarily on the basis of being heavy smokers who also showed the outward appearance of hyperthyroidism. Strauss and Scheer[161] tested the effect of smoking eight to twenty cigarettes on the blood iodine of smokers and nonsmokers and stated that two types of blood iodine curves were obtained: type I, correlated with occasional smokers, nicotine-sensitive individuals, and hyperthyroid patients, showed an increase in blood iodine with maximum values (50 to 100 per cent above controls) appearing in about 60 minutes and returning to normal levels in 120 to 180 minutes; type II, correlated with habitual smokers and hypothyroid patients, showed a decrease in blood iodine with minimum values (25 to 50 per cent of controls) appearing in about 60 minutes, with return toward normal levels in about 180 minutes. Gutzeit and Parade[54] postulated that an increase in epinephrine secretion might be the mechanism of the rise in blood iodine observed after smoking. These latter authors reported that the smoking of a cigarette caused a rise in total blood iodine in fasting, resting nonsmokers, the greatest increase appearing in the organic iodine content, sometimes to double the original level. A definite drop toward the normal level occurred within 1 hour. With habitual smokers, smoking a cigarette caused only a slight increase in

total and in organic iodine. Exercise was said to cause as great an increase in blood iodine as smoking.

Strauss and Scheer[161] also studied the effect on blood iodine of acute and chronic administration of nicotine to dogs and rabbits. As in the case of smokers described above, two types of blood iodine curves were said to have been obtained in both acute and chronic nicotine-treated animals.

Magnesium. Blood magnesium levels were found to be uninfluenced by the smoking of three cigarettes in succession (Högler[70]).

Bromine. In the normal dog, the amount of blood bromine was decreased by the injection of nicotine, and hypophysectomy did not markedly alter the effect of nicotine upon the bromine levels (Saito[141]).

Sulfur. Saito[140] reported that sulfur metabolism in rabbits was decreased by daily injection of 0.1 mg. nicotine tartrate per kilogram.

The total amount of sulfuric acid excreted by smokers was said to be 2.6003 Gm. per day, and that of nonsmokers, 2.5362 Gm. (Tsuru and colleagues[177]). The total amount of ethyl sulfuric acid excreted by smokers was 0.1886 Gm. per day, 16 per cent more than that of nonsmokers.

Vitamin metabolism

In reply to a question recently submitted to the *Journal of the American Medical Association* (168:2334, 1958) concerning the effect on vitamin metabolism, especially that of the B complex group and vitamin C, in the human body in an individual who smokes and inhales twenty to forty cigarettes a day, the consultant stated that he was unaware of any evidence indicating that cigarette smoking has an adverse effect on the body's ability to utilize vitamins. Whether the work described below constitutes evidence or not, it is nevertheless now an imperishable part of the Tobacco Literature.

Thiamine. A constant and marked reduction in urinary excretion of thiamine was observed in a few subjects after they smoked one to three cigarettes (Strauss and Scheer[162]).

Ascorbic acid. In a study of 30 men and women aged 18 to 40 years, Strauss and Scheer[162] found a constant and marked reduction in urinary excretion of ascorbic acid after smoking of one to three cigarettes. Following experiments which may be characterized as unsatisfactory, Goyanna[51] claimed that no urinary elimination of the vitamin could be ascertained in those who smoked more than twenty cigarettes a day, while in those smoking up to ten cigarettes, there was excretion of ascorbic acid; in subjects smoking ten to twenty cigarettes, excretion was said to be delayed.

Harmsen[60] noted that 9 subjects who had abnormally low blood ascorbic acid levels were also heavy users of tobacco; but whether or not there were other heavy smokers who did not show this effect was not apparent from his report. In studies on 60 medical students, the ascorbic acid in the blood of nonsmokers was found to range between 1 and 1.2 mg. per 100 ml., and in smokers between 0.6 and 0.9 mg. per 100 ml. (Venulet and Moskwa[185]; Venulet[180]). In nonsmokers smoking eight cigarettes a day, a decrease in blood ascorbic acid was noted after 3 days, with return to normal in 5 days after cessation of smoking. Venulet[180, 181] suggested that the deficiency in ascorbic acid induced by smoking might be responsible for a variety of ill-effects, such as interference with brain and muscular activity and damage to the adrenal glands, liver, and kidneys, and even for the development of certain character traits of smokers.

Bourquin and Musmanno[14] also reported that smoking could lower the ascorbic acid content of the blood. In 3 persons, smoking respectively about one-sixth pack a day, about one pack without consciously inhaling, and one and one-half packs a day with inhalation, the ascorbic acid content of the blood during a 3 to 4 day period of unrestricted diet and smoking was 1.03, 0.62, and 0.44 mg. per 100 ml. respectively. On a

restricted diet supplemented by oral administration of 75 mg. ascorbic acid per day, the ascorbic acid content of the blood was, respectively, 1.02, 0.70, and 0.51 mg. per 100 ml. in a 4 day nonsmoking test period and 0.85, 0.62, and 0.17 mg. per 100 ml. in a smoking period.

In contrast to other workers, Högler[70] reported that studies on more than 100 smokers and nonsmokers revealed no regular differences between fasting ascorbic acid blood levels of smokers and nonsmokers, even in acute tests (smoking three cigarettes in succession, fasting).

Venulet and Danysz[182] stated that the content of ascorbic acid in the milk of smoking mothers was less than that in the milk of nonsmoking mothers, while the increase of ascorbic acid in the milk of smoking mothers who took this vitamin internally was considerably less than in the case of nonsmoking mothers. Although large fluctuations, depending on diet, season, sickness, body temperature, and personal peculiarities, were observed, the milk of 28 nonsmoking women was found to contain 2.8 times as much vitamin C as that of 10 smokers; in the autumn, however, it was only 1.4 times as much (Venulet and Danysz[183]). The degree of difference was said to be related to the number of cigarettes smoked, and, on stopping smoking, the vitamin C level rose sharply. After daily ingestion of 500 mg. of vitamin C over a period of 3 to 4 weeks, its level rose in the milk of 2 nonsmokers from a pretreatment level of 5 mg. per 100 ml. to 6.3, and in 2 smokers, from an initial level of 2.4 to 3.6 mg. per 100 ml.

In experiments on mice exposed to an atmosphere of cigarette smoke for 20 minutes a day, there was said to be a 100 per cent increase in urinary excretion of ascorbic acid on the day after the first exposure (Venulet and Moskwa[185]; Venulet[180]). Thereafter, a progressive decrease below normal set in: after three exposures, the decrease was 10 to 20 per cent; after 1 week's exposure, up to 50 per cent; and after 4 months' exposure, 90 to 95 per cent. Return to normal levels occurred in 3 days after one exposure, in 5 days after three exposures, and in 8 days after 1 week's exposure. In mice given 20 mg. ascorbic acid orally per day, urinary ascorbic acid increased to 300 per cent of normal in control animals, while in the experimental group exposed to tobacco smoke over 8 days, the urinary level dropped to 50 per cent of its initial value. Tissue studies indicated that adrenal ascorbic acid was responsible for the initial elevation; and it was concluded that tobacco smoke caused an initial mobilization of ascorbic acid, together with an increased decomposition of the vitamin. Strauss and Scheer,[162] it may be noted, regarded the effect of smoking on the urinary excretion of ascorbic acid (as well as of thiamine) as related to an action on the thyroid and adrenal glands and, through some influence on the sympathetic nervous system, leading to an enhanced level of general oxidative activities.

Wenzel and Beckloff[198] were unable to demonstrate significant differences in the serum ascorbic acid of rabbits maintained on a control diet, fed 0.1 per cent cholesterol in the diet, given 2.8 mg. nicotine per kilogram per day in the drinking water, or given combined cholesterol and nicotine.

In vitro, the destruction of ascorbic acid by nicotine proceeded only slowly in pure distilled water but could be markedly accelerated by the presence of small amounts of copper (Reif[136]). The reaction proceeded rapidly in a slightly alkaline medium but was inhibited in an acid one (e.g., pH 4). With small amounts of nicotine, the reaction stopped in part at the dehydroascorbic acid stage; with larger amounts, no ascorbic acid was recoverable by treatment with hydrogen sulfide. Bourquin and Musmanno[13] added to blood of known ascorbic acid content nicotine in amount estimated to equal the amount of nicotine which might be absorbed in heavy smoking; in three experiments, they found that 24.4 to 31.6 per cent of the original ascorbic acid had been lost after 5 or 10 minutes. Goyanna[51] macerated cigarettes,

added ascorbic acid to the filtrate until a color reaction could be produced by a reagent (dichlorophenyl-indophenol sodium), and reported that 2 mg. ascorbic acid was used up by each cigarette. In this connection, McCormick[117, 118] stated he had determined by laboratory and clinical tests that the smoking of one cigarette neutralizes in the body approximately 25 mg. ascorbic acid.

Regarding the ascorbic acid content of the adrenal glands, Maren[112] examined the effect of low doses of nicotine on adrenal ascorbic acid in normal and hypophysectomized male rats and found a statistically significant decrease after nicotine treatment. Hypophysectomy abolished this effect, and it appeared that nicotine, like a number of other drugs, stimulated the adrenal glands by a pathway which included the adenohypophysis. (With respect to mechanism, Vogt[187, 188] noted that, in the intact animal, nicotine is bound to increase adrenal cortical secretion by raising the epinephrine level in the circulation. There appears to be no direct action on cortical tissue, since nicotine had no effect on the rate of secretion of adrenal cortical hormone in perfused adrenal glands of dogs.) In frogs exposed to tobacco smoke, adrenal ascorbic acid was initially depleted at a time when blood ascorbic acid levels were greatly increased (Venulet and Moskwa[185]; Venulet[180]); and mice exposed to tobacco smoke for 5 to 10 minutes daily for 20 days showed a considerable decrease in the ascorbic acid content of the adrenal glands (Venulet and Majcherski[184]). (In acute experiments reported by De Schaepdryver,[27] prior administration of iproniazid to mice prevented nicotine depletion of adrenal ascorbic acid.) Venulet and Majcherski[184] also studied the effect of chronic tobacco smoke exposure on the functional state of the adrenal cortex and claimed to have demonstrated disturbances in the function of the adrenal gland under the influence of a "stress" factor, such as tobacco smoke, leading to a weakening of resistance.

Judging from progressive reductions in adrenal ascorbic acid content and in eosinophils, Lupu, Velican, and Mihaescu[108] assumed that in guinea pigs and rabbits exposed to cigarette smoke, an important secretory response had been produced in the adrenal cortex. Histochemical examination of the glands for ascorbic acid corresponded to the biochemical findings.

It may be noted in passing (for what it is worth) that there appear to be no reports in the clinical literature suggesting that smoking is in any way related to adrenal cortical function or dysfunction.

Organ and tissue metabolism

Having discussed intermediary metabolism in the intact organism, we shall now proceed to canvass the effects of nicotine on intermediary metabolism successively in organs, tissues, suspensions of cells, and extracts prepared from cells.

Heart. In anesthetized dogs, nicotine increased left ventricular oxygen consumption (Schmitthenner and coauthors[149, 150]). In the open chest dog administered pentobarbital and made to inhale 1,200 to 1,500 ml. of cigarette smoke, the coronary arteriovenous oxygen difference decreased markedly at first, and this was followed by a prolonged increase (Kien, Lasker, and Sherrod[87]). As a consequence, initially the cardiac oxygen utilization was reduced during the period of a greatly elevated cardiac work. This decrease was followed by a sustained increase, and it was suggested that these alterations might be explained on the basis of metabolic changes in the myocardium.

In studies on human subjects smoking one cigarette, myocardial oxygen usage was not significantly changed; glucose usage was not significantly increased; pyruvate extraction fell, but the change was not statistically significant; myocardial extraction of ketones rose after smoking, although not significantly (Bargeron and associates[6]).

Using Starling's heart-lung preparation, Kinoshita[89] found that nicotine increased the oxygen consumption of the heart for a

short time after injection but then decreased it; in most cases, augmentation of the oxygen consumption of the heart ran parallel to dilatation of the coronary vessels. Employing the Warburg technique, Holz and Rossi[73] reported that 1:714 nicotine added to a thin strip of rhythmically beating right rabbit auricle (connected with the region of the S-A node) produced a more or less considerable increase (up to 200 per cent) of the oxygen consumption during the first few minutes, followed by a rapid decrease of the respiratory activity to a level decidedly lower than the initial value. On the other hand, the oxygen consumption of the immobile left auricle was not at all influenced by the action of nicotine.

Glucose metabolism by heart muscle was said to have been stimulated by nicotine and also by atropine, and addition of the two drugs together produced an additive effect (Gitter[48]).

Brain. During the excitement following intravenous injection of nicotine into rabbits, Yamakita[206] observed an acceleration of oxygen consumption in the brain with increased blood flow, and in the ensuing condition of rest, a decrease in both. In mice in nicotine convulsions, brain lactic-acid values did not differ significantly from those of normal animals (Stone[159]).

In studies on man, no significant changes in cerebral blood flow, oxygen consumption, vascular resistance, respiratory quotient, blood pH, or gases were observed in normal subjects after the smoking of three cigarettes (Wechsler[197]). Intravenous injection of 8 to 10 mg. nicotine over a 30 minute period resulted in a statistically significant increase in cerebral metabolism in 3 of 5 subjects, but this increase may have been due to the observed side reactions rather than to the nicotine itself.

According to Gitter,[48] nicotine stimulated metabolism of sugar by brain tissue. As determined by the Warburg technique, 0.014M nicotine did not inhibit oxidation of glucose in the brain of normal rats, cats, and dogs or in brain tissue from diabetic cats and dogs (Fazekas and Himwich[39]). Aerobic glycolysis in brain slices was greatly accelerated by the presence of nicotine (Quastel and Wheatley[134]; Baker, Fazekas, and Himwich[5]; McIlwain and Grinyer[121]). Such concentrations of nicotine as increased aerobic glycolysis had little effect on the tissue respiration, however (McIlwain and Grinyer[121]; Case and McIlwain[20]).

The effect of nicotine on oxidation in brain tissue has been extensively studied by Himwich and his co-workers.[4, 5, 39, 68] Nicotine in 0.014M concentrations caused a 60 per cent inhibition in the oxidation of lactic acid but did not inhibit oxidation of glucose in brain slices of normal or diabetic animals. The oxygen uptake caused by pyruvic acid, the first stage in the oxidation of lactic acid, was not inhibited by nicotine; there was, in fact, a 15 per cent stimulation; and it was therefore deemed probable that the inefficiency of lactic acid oxidation was due to the action of nicotine on a dehydrogenase of lactic acid. The respiratory quotient disclosed that glucose and pyruvic acid were completely oxidized, while combustion of lactic acid was inhibited. From some of their studies, Himwich and Fazekas[68] concluded that nicotine not only prevented the oxidation of lactic acid but also its formation. Later Baker and Himwich[4] reported from quantitative studies that 0.03M nicotine caused practically complete inhibition of lactate oxidation, and this concentration of nicotine stimulated the aerobic formation of lactic acid, which, since it was not oxidized, accumulated. The decrease in glucose could be accounted for by the increase in lactic acid and the oxygen consumption: glucose was therefore oxidized without passing through an intermediary lactic acid stage. A quantitative study of the metabolism of slices of cerebral cortex of rats and of normal and pancreatectomized cats in the Dixon-Keilin apparatus showed that in the presence of a concentration of nicotine which prevented the oxidation of lactic acid, the disappearance of

glucose could be accounted for by the oxygen consumption (corrections being applied for glucose going to form lactic acid). Nicotine inhibited the oxidation by cerebral tissue of various normal substrates to different degrees: pyruvate and lactate were inhibited to about the same extent, to a much greater degree than glucose; fructose was also inhibited more than glucose; succinate oxidation was practically unaffected.

The respiration of rat cerebral cortex was decreased by 10 per cent in 0.005M nicotine and by 22 per cent in 0.01M nicotine (Yamamoto and Kurokawachi[207]).

In examining the possibility that the toxicity of nicotine to nerve cells might be attributable to its ability to block essential steps in the oxidative metabolism of carbohydrates, Fahmy and Walsh[34] made an analytic study of the effect of nicotine on the oxidation of pyruvate through the citric acid cycle. Using homogenates of rat brain cerebral cortex, pigeon cerebral hemispheres, pigeon liver, or rat kidney cortex, they studied the effects of nicotine on (1) respiration of brain tissue with glucose and pyruvate as substrates, (2) oxidation of succinate in brain, (3) oxidation of fumarate to oxaloacetate in kidney, (4) oxidation of citrate to α-oxoglutarate in liver, (5) oxidative decarboxylation of α-oxoglutarate in brain, (6) citrate synthesis in brain and kidney, and (7) oxidative decarboxylation of pyruvate. In confirmation of Himwich and co-workers, the oxidation of pyruvate was found to be more sensitive to nicotine than was that of glucose. Inhibition of citrate formation by nicotine (step 6) was found to be greater than that of any of the other steps in the citric acid cycle, and inhibition of the pyruvate dehydrogenase system (step 7) occurred to the same extent. Fahmy and Walsh therefore concluded that nicotine inhibits pyruvate oxidation in brain by specifically inhibiting pyruvic dehydrogenase, other oxidative steps being less sensitive. Addition of thiamine in concentrations equal to that of nicotine did not modify the effect of nicotine on the pyruvic dehydrogenase system in dialyzed pigeon brain homogenate.

In calf brains from which a brei was made, 1:1,000 nicotine accelerated the rate of formation of free phosphoric acid on an average of 3.5 per cent (Stamm[155a]). Lowered phosphorylation, with little if any change in respiration, was observed to follow the addition of nicotine in concentrations of 0.01M to 0.001M to nondialyzed homogenates of guinea pig brain (Case and McIlwain[20]).

In studying the tissue uptake of substances added to slices of guinea pig brain cortex shaken aerobically in glucose-saline, McIlwain and Grinyer[121] found that nicotine was largely excluded from the slices, in contrast to certain phenazine and quinoline derivatives, which were absorbed until their concentrations in the slices were fifty to three hundred times those in the solutions.

The effect of nicotine on brain cholineacetylase and cholinesterase is described below.

Yamamoto and Kurokawachi[207] gave rats subcutaneous injections of nicotine over periods of 60 or 100 days, then made tissue respiration measurements by the Warburg technique on cerebral cortex in 0.005M and 0.01M nicotine; but no appreciable difference was found in the respiration of brain slices from normal control or chronic nicotine-treated animals. Takeuchi, Kurogochi, and Yamaoka[165] also found no distinct difference in tissue respiration of the cerebral cortex of rats given daily intramuscular injections of convulsive doses of nicotine for 30 to 120 days and in that from normal animals.

Liver. Nicotine was said by Gitter[48] to stimulate the dehydrogenation processes in liver tissue; but, according to Fahmy and Walsh,[35, 36] nicotine inhibited glucose dehydrogenase of mammalian liver.

Stewen[158] studied the effect of nicotine on the formation and degradation of hexosediphosphate and glycerophosphate in tissue breis of liver, skeletal muscle, and

kidney of rabbits or cattle and reported that nicotine inhibited the formation and the degradation of organic phosphates in tissue.

Werle and Müller[202] measured oxygen consumption of liver and lung slices in the presence of nicotine by the Warburg method and found that small amounts of nicotine increased oxygen consumption, whereas larger amounts decreased it. The respiration of liver slices from normal rats was increased by 5 per cent in 0.005M nicotine and by 33 per cent in 0.01M nicotine (Yamamoto and Kurokawachi[207]).

Tissue respiration of liver slices from chronic nicotine-injected rats showed no distinct or consistent differences from liver slices of normal control animals, whether measured in the absence or presence of nicotine (Yamamoto and Kurokawachi[207]; Takeuchi, Kurogochi, and Yamaoka[165]).

Kidney. In kidney and in testes, nicotine inhibited glucose and lactic acid oxidation to the same extent, in contrast to the results in brain tissue, in which nicotine markedly inhibited the oxidation of lactic acid but did not inhibit oxidation of glucose (Baker, Fazekas, and Himwich[5]). The aerobic glycolysis of kidney and testes was not accelerated by nicotine, again in contrast to the effect on brain.

The activity of malic dehydrogenase in kidney was unaffected by nicotine in 0.0075M concentration and was inhibited by about 12 per cent by 0.03M nicotine (Fahmy and Walsh[36]).

Respiration of kidney slices from normal rats was decreased in the presence of nicotine (Yamamoto and Kurokawachi[207]); but respiration of kidney slices from chronic nicotine-injected animals did not differ appreciably or consistently from respiration of tissue slices from normal control rats (Yamamoto and Kurokawachi[207]; Takeuchi, Kurogochi, and Yamaoka[165]).

Lung. Kolberg[95] studied oxygen uptake by minced rat lung tissue in the presence of cigarette smoke solution and reported that test and control tissues respired comparably until 42 minutes later, at which time the treated tissue decreased in oxygen utilization rate. When cigarette smoke was forced into the interior of freshly excised rat lung prior to mincing, the subsequently minced tissue respired comparably to control tissue for 55 minutes, after which the rate for the smoke-treated tissue somewhat declined. In a third (and, to the author, more satisfactory) procedure, anesthetized rats were made to inhale cigarette smoke via a tracheal tube; subsequently minced lung tissue respired at a slower rate than that of controls. These findings were said to indicate that the metabolic machinery of the lung tissue cells, namely the minute mitochondria, had been affected: tobacco smoke may have altered the permeability of the plasma membrane, thus hindering the diffusion of oxygen through it, or else the alkaloidal nature of tobacco smoke may have affected the riboproteins of the cytoplasm, or both.

Muscle. During nicotine contracture of frog gastrocnemius, a twofold to threefold increase in oxygen consumption occurred (Zondek and Matakas[208]). (Only a slight increase in the rate of anaerobic glycolysis was found.) In concentrations which caused contraction of frog sartorius muscle, nicotine led to increased oxygen consumption, although an exact correspondence between the threshold for these two phenomena was not demonstrable (Fenn[40]).

The activity of lactic dehydrogenase in muscle was unaffected by 0.0075M nicotine and somewhat inhibited by 0.03M (Fahmy and Walsh[36]).

Gitter[48] studied the rate of dehydrogenation of different types of muscle after the addition of nicotine and atropine. In low concentrations, nicotine inhibition of metabolism was said to be lessened by atropine; in high concentrations, atropine increased the toxic action of nicotine.

A very brief account of some effects of nicotine on the chemistry and metabolism of muscle will not be irrelevant here. After injection of nicotine, muscle glycogen content markedly decreased in frogs (Kato[85, 86]) and rabbits (Hiraoka[69]). A

parallelism between the degree of nicotine contracture and lactic acid production in frog gastrocnemius was observed by Matsuoka,[114] but Zondek and Matakas[208] concluded there was no causal relation between the two phenomena. The contracture produced by nicotine in denervated mammalian muscle was accompanied by the production of lactic acid of the same magnitude as that observed in tetanus-producing equivalent tension (Gasser and Dale[47]).

According to Holl,[72] nicotine inhibition of lactic acid formation in mammalian muscle could be annulled by appropriate concentrations of atropine.

During nicotine contracture, the creatine content of frog muscle was reportedly increased (Mitsuda and Uyeno[123]) or unchanged (Nishimura[130]; Kato[85, 86]). After subcutaneous injection of nicotine in rabbits, the creatine content of the vastus lateralis muscle showed a 2.4 per cent increase (Hiraoka[69]). The ratio of bound creatine to total creatine was less than before injection, as Kato[85, 86] also found in frogs.

In Hiraoka's rabbits, the muscle phosphate content was found to be increased 25 to 60 minutes after the nicotine injection, the increase paralleling that in bound creatine and inversely proportional to phosphogen. In Kato's frogs also, muscle phosphogen phosphate decreased markedly, while inorganic phosphate increased. In experiments on isolated frog muscle, creatine phosphate decreased following nicotine contraction, but there was no change in adenosinediphosphate or adenosinetriphosphate (Fleckenstein and associates[42]; Janke[82]).

Testis. In the testes, nicotine inhibited glucose and lactic acid oxidation to the same extent; the aerobic glycolysis was not accelerated (Baker, Fazekas, and Himwich[5]).

Blood cells. Konishi[96] reported that the threshold concentration of nicotine hydrochloride producing inhibition of respiration of blood was 0.000001M.

Enzyme systems: Cholinesterase and cholineacetylase. Nachmansohn[126] demonstrated that nicotine possessed anticholinesterase activity, and this observation has been confirmed in various experimental setups by several other workers (Koelle[93]; Bain[3]; Todrick[171]). In guinea pigs killed with cigar smoke, Werle and Meyer[201] found a 50 per cent inhibition of brain cholinesterase; the nicotine concentration in the brain was about 15 mg. per kilogram.

Orgell, Vaidya, and Dahm[131] reported that a filtered homogenate of the leaves of *Nicotiana tabacum* inhibited the cholinesterase activity of human plasma. This inhibitory substance does not appear to be related chemically to the better known tobacco alkaloids.

Nachmansohn and Schneemann[128] studied the inhibitory effect of central nervous system stimulants on four enzyme preparations, two representing specific cholinesterase and two unspecified esterases. Nicotine weakly inhibited both types of esterases. Only caffeine and theobromine were found to act exclusively on cholinesterase, which the authors pointed out as an interesting fact in view of their action as general stimulants of the central nervous system.

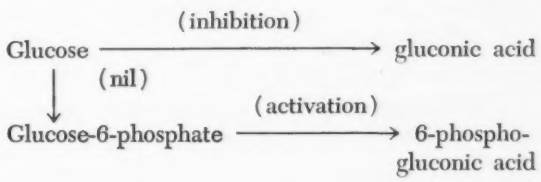
In pharmacologic opposition to the ability of nicotine to inhibit acetylcholine breakdown is the action of nicotine tending to suppress acetylcholine synthesis. Fahmy, Ryman, and Walsh[37] found that 0.015M nicotine produced about a 40 per cent inhibition in the activity of cholineacetylase prepared from rabbit brain. This concentration of nicotine had no effect on the sulfonilamide-acetylating system of pigeon liver. This was taken to indicate that nicotine is not a general inhibitor of all mechanisms in which active acetate is involved and that its action on cholineacetylase is a specific one. While the effect on cholineacetylase is not in itself sufficiently high to be of marked pharmacologic significance, it must be borne in mind that nicotine also inhibits the reaction whereby active acetate is produced from pyruvate (see above),

and in all probability, nervous tissue depends, primarily if not exclusively, on pyruvate as the acetyl donor for choline acetylation (see Fahmy and Walsh,[34] above). Since nicotine inhibits the initial step in pyruvate oxidation and, at the same time, stimulates glycolysis, the tendency for the local concentration of pyruvate to rise above the normal level would be an additional factor contributing to the inhibition of acetylcholine synthesis. Nachmansohn and John[127] have shown that pyruvate is a powerful inhibitor of choline-acetylase. Evidence has also been presented that nicotine interferes with coupled phosphorylation (Case and McIlwain[20]), thereby adding to the effects that follow from inhibition of the energy-yielding mechanism of carbohydrate oxidation. Under physiologic conditions, therefore, the inhibitory action of nicotine on acetylcholine synthesis would be expected to be considerably greater than that which results from direct action of the drug on choline-acetylase.

Dehydrogenases. Harris and Creighton[61] reported that nicotine prolonged the time required by reductase of liver juice to reduce oxyhemoglobin to the one-banded condition. Gitter[48] found that nicotine was a stimulant to the dehydrogenation processes in liver tissue.

Direct observation of the time required for the decolorization of methylene blue by brain slices showed that nicotine interfered with dehydrogenation; and Himwich and Fazekas[68] considered it probable that the insufficiency of lactic acid oxidation in brain slices was due to the action of nicotine on a dehydrogenase of lactic acid.

Fahmy and Walsh have made extensive studies on the effect of nicotine on dehydrogenase systems. Nicotine in 0.03M concentration had no effect on the activity of either isocitric dehydrogenase, xanthine oxidase, or the Schardinger enzyme of milk; succinic dehydrogenase was but slightly inhibited (Fahmy and Walsh[36]). The activities of lactic dehydrogenase in brain and in muscle, and of malic dehydrogenase in kidney, were unaffected by 0.0075M nicotine, while 0.03M inhibited the systems by about 12 per cent. Nicotine inhibited glucose dehydrogenase of mammalian liver (Fahmy and Walsh[35]); 0.0075M inhibited this system by about 16 per cent (Fahmy and Walsh[36]). Increasing the concentration of diphosphopyridine nucleotide in this system did not modify the inhibitory action of nicotine. The pyruvic dehydrogenase system in rat and pigeon brain homogenate preparations was inhibited by nicotine, and additions of thiamine in concentrations equal to that of nicotine did not modify the nicotine effect (Fahmy and Walsh[34]). The alcohol dehydrogenase system of yeast was activated by 0.0075M nicotine by about 30 per cent (Fahmy and Walsh[36]). But whereas 0.01M nicotine inhibited dehydrogenase activity of *Saccharomyces cerevisiae* up to 20 per cent with glucose as substrate, activations up to 40 per cent resulted with glucose-6-phosphate (Fahmy and Walsh[35]). A similar inhibition of nicotine was found for glucose dehydrogenase of *Torulopsis utilis.* With a partially purified hexokinase prepared from yeast, this concentration of nicotine had no effect on the transfer of phosphate from adenosinetriphosphate to glucose. Fahmy and Walsh[35] summarized these latter nicotine effects in this schema:



Fahmy and Walsh[36] commented on the above findings as follows: From the experiments with isocitric, xanthine, acetaldehyde, and succinate as substrates, it may be inferred that nicotine does not interfere with the electron-transfering mechanism through the yellow enzymes and cytochrome system. Of the enzyme systems studied, those which are appreciably affected by nicotine, with the possible

exception of cholineacetylase, are the dehydrogenases which require a pyridine nucleotide as coenzyme. Nicotine also inhibits the enzymes for which diphosphopyridine nucleotide (cozymase or cozymase I) is the substrate (McIlwain[120]). Since nicotine, like the nicotinamide moiety of the coenzymes, contains a β-substituted pyridine ring, one might expect nicotine competitively to inhibit all the dehydrogenases which require one or another of the pyridine nucleotides. Isocitric dehydrogenase, however, is unaffected by nicotine, while glucose-6-phosphate dehydrogenase is activated. Both these enzymes require the triphosphopyridine nucleotide. On the other hand, lactic, malic, pyruvic, and glucose dehydrogenase, all of which require diphosphopyridine nucleotide, are inhibited by nicotine. From the experiments with glucose dehydrogenase, however, it would appear that inhibition is not competitive with regard to the coenzyme. Alcohol dehydrogenase, which also requires diphosphopyridine nucleotide, is strongly activated by nicotine to an extent which might well contribute to the stimulant effect of nicotine on alcoholic fermentation. Fahmy and Walsh felt it necessary to conclude, therefore, that while there is a tendency for nicotine to interfere with enzyme systems which involve the pyridine nucleotides, the nature of the effect is unpredictable and is characteristic of the individual dehydrogenase system, rather than that of the group to which it belongs.

Cozymase. Nicotine in concentrations of about 0.001M in preparations of guinea pig and sheep brain and ox spinal cord inhibited by 50 per cent the breakdown of 0.0006M cozymase (McIlwain[120]; McIlwain and Grinyer[121]).

Miscellaneous observations on enzymes. Nicotine in 0.01M concentration had no inhibitory effect on myokinase and adenosinetriphosphate (Carr and colleagues[19]).

The histaminase level in the blood of smokers showed no increase, as compared to that of nonsmokers (Werle and Effkemann[200a]).

Conclusions

Possibly the most important aspect of this review of the effects of nicotine (and smoking) on metabolism is the revelation of the gaps in our knowledge of the ultimate action of "pharmacologic" doses of nicotine. But this is not necessarily a trumpet call to rush in and fill the void; for the cellular pharmacodynamics of nicotine (if we may thus express these ultimate actions) can advance no further or faster than knowledge of the biochemistry and biophysics of the "physiologic" cell; and it would be idle to keep on adding a dollop of nicotine to some artificial and arbitrary "system," as many of the older workers used to do, and expect the "result" to be meaningful. All we can suggest at this time with respect to future research is to caution the investigator that so potent a drug as nicotine can be *made* to have an influence on metabolism, whether in the intact organism, individual organs, tissues, cells, or cellular components—*when used in sufficient quantity.* As a corollary, it may be emphasized that it is the concentration of nicotine obtaining in the cells or intracellular fluid of normal smokers which alone carries any validity in assessing the effects of tobacco smoking. In a word, concentration is all, and "experimental" concentrations of nicotine must be matched to those "normally" occurring in tissues and body fluids of smokers. Only such concentrations can elucidate the *pharmacologic aspects* of tobacco smoking: concentrations many times these express only the *toxicologic characteristics* of nicotine. We may, however, point out that if toxic doses or concentrations of nicotine have no demonstrable effect on metabolic—or, indeed, on other—activities, this *negative* evidence is very probably valid with respect to tobacco smoking; whereas the converse is, equally probably, far from true. It is never a work of supererogation to repeat that tobacco smoking, as it is performed by the smoking millions, is a pharmacologic, and only very rarely indeed a toxicologic phenomenon.

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Drug therapy in hypertension

The methods available for the reduction of the blood pressure level in arterial hypertension are outlined.

Effective blood pressure reduction decreases considerably the mortality of patients with grades 4, 3, and 2 retinopathy.

In 1961, very few hypertensive patients should continue to complain of potentially reversible hypertensive symptoms or of distressing side effects, although it may be necessary to try several drugs or combinations of drugs.

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This article presents an attempt to escape from detail—to discover what in general the aim should be when confronted with a hypertensive patient and, when a decision has been made to treat, how a choice should be made among the hypotensive agents available. The detail of how to get the best out of the drugs that are available is beyond the scope of this article, but reference is made to convenient summaries.

In the last 10 years well over 1,000 patients with hypertension have been investigated in our clinic. The statements made are here based largely on the experience gained thereby.

The progress in the pharmacology of hypotensive drugs has led to a situation in which most patients attending our clinic for 5 to 10 years have had experience with several drugs. However, the aim of exploring the degree of benefit obtainable by return of the blood pressure to a near normal level for as much of the 24 hour day as can be managed remains unchanged. Before hexamethonium was available, the view had been expressed that hypertension as such led to many, probably most, of the manifestations characteristic of hypertensive disease[21]; therefore, reduction of the arterial pressure should be accompanied by improvement. Many substances were screened pharmacologically for hypotensive activity and a number of chemical substances were given a clinical trial and rejected. When potent drugs became available, the experience gained was found to have been worth while. From the beginning, several patients with either malignant hypertension or hypertensive heart failure were treated with a continuous subcutaneous injection of hexamethonium by use

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of a mechanically operated syringe to maintain the blood pressure at a normal or near-normal level for days without intermission.[24] A laboratory assistant measured the blood pressures at 15 minute intervals throughout the day, and resident medical officers made some measurements during the night. The results obtained were objective and dramatic and provided the justification for long-term treatment in which we aimed at obtaining normal or near-normal blood pressure in most of the patients without severe discomfort to them. To have most patients comfortable is, in 1961, a matter of technique; to get the best results, careful clinical trials are needed that amount to a biologic assay, usually lasting a week or so, but sometimes lasting months.

On theoretic grounds, it was thought that the reduction of blood pressure should be applied not only to patients with grade 4 and grade 3 retinopathy but also to those with grade 2 changes for preventive reasons. In the 11 years since January, 1950, we have gained experience which enables conclusions to be drawn about the effect of blood pressure reduction on the outlook of hypertensive patients who have no exudative retinal changes. Once a decision was made that a patient's blood pressure should be reduced, even in the milder cases the aim was at reduction to near normal levels.

When hexamethonium given subcutaneously was the only conveniently available and effective agent, parasympathetic side effects were difficult to avoid and various measures were devised, such as the combination of hexamethonium with salt restriction (now unnecessary) and the administration of parasympathetic stimulants.[22] With the use of the newer drugs (pentolinium, Rauwolfia alkaloids, mecamylamine, pempidine and its homologues, chlorothiazide, hydrochlorothiazide, bretylium tosylate, and guanethidine) it has become possible to obtain a good control over hypertension, with very few serious side effects.

Selection of patients for hypotensive therapy

It is generally agreed that prognosis in the absence of treatment is very poor in hypertensive patients with such changes in the retina as papilledema, retinal edema, retinal hemorrhages, or soft or hard exudates. Of untreated grade 4 patients who have papilledema, less than 50 per cent survive 6 months.

Although the outlook of grade 4 patients is much better* [11, 23, 27] with effective blood pressure reduction, it is still much worse than the prognosis of patients who have not progressed to this advanced stage. *My impression is that it is most exceptional for grade 4 changes to develop in a patient whose blood pressure has been reduced effectively at an earlier stage. It is my belief that the practice of waiting for advanced changes to develop before undertaking treatment is responsible for avoidable disability and loss of life.*

Hypotensive therapy is generally accepted as essential in patients with hypertensive heart failure or cardiac asthma of whatever retinal grade. Effective reduction of the blood pressure level has almost eliminated uncomplicated heart failure as a cause of death in hypertensive patients.[2, 25, 28, 33] Heart failure, however, remains as a cause of death in hypertension complicated by advanced renal failure. It has been our practice from the outset to give hypotensive therapy to hypertensive patients with such manifestations as substantial breathlessness on exertion, appreciable cardiac enlargement, electrocardiographic indications of cardiac hypertrophy, severe headache, and substantial hypertensive giddiness.

An 8 year follow-up of our grade 2 patients on treatment and grade 2 control individuals has now yielded statistically significant figures ($P < 0.001$) indicating reduced mortality in the former.* The mortality of grade 2 patients on hypotensive

*J. V. Hodge, E. G. McQueen, and F. H. Smirk: Unpublished data.

treatment is about half that of untreated controls; the incidence of heart failure is negligible. Even if we do not consider all grade 2 and control patients presenting with congestive heart failure or cardiac asthma, the decrease in the mortality of treated patients remains significant, which indicates that the reduced mortality is not solely dependent on the now well-known improvement in heart failure patients.

While many clinics agree that severe grade 2 patients should receive treatment, there is less agreement about treatment in the group of symptomless patients with hypertension. At our clinic the latter is subdivided into two groups: patients with high and patients with low basal blood pressures. The basal blood pressure is more reliable as an index of severity than is the supplemental blood pressure. Indeed, the supplemental blood pressure (i.e., casual minus basal pressure) has little influence upon an 8 year prognosis.[29] By our technique, basal blood pressures are much lower than casual blood pressures.

Details of the method used to determine the basal blood pressure have been described elsewhere.[13, 20] In brief, the procedure consists of reassurance of the patient, who rests overnight in a single room after taking a sedative, usually 90 to 180 mg. of pentobarbital. A further 90 mg. of pentobarbital is given in the morning unless the patient is drowsy. The bowels and bladder are emptied if desired. In order to remove the effect of emotion on blood pressure, a further ½ hour rest is permitted and then the blood pressure is measured repeatedly at ½ to 1 minute intervals, in a room without disturbance of any kind, for a period of not less than 20 minutes.

The average of the lowest two pressures is the basal blood pressure. Less exacting procedures lower the blood pressure but not to the basal level. Basal blood pressures determined by the practitioner in the patient's own home will often be more reliable than those in hospital.

Our mortality findings in patients with untreated hypertension are shown in Table I. The mortality in men began to rise appreciably in those with *basal* systolic pressures above 140 mm. Hg and *basal* diastolic pressures above about 80. The mortality in women began to rise appreciably when basal systolic blood pressures were above 150 mm. Hg or the basal diastolic blood pressures were above 90. It would appear from these 8 year follow-up results on untreated or substantially untreated patients that close consideration should be given to the treatment of women whose basal blood pressures exceed 150/90 mm. Hg or to men whose basal blood pressures exceed 140/85.

There is also the question of whether patients with mild, symptomless hypertension, with a casual blood pressure of, say, 170/90 mm. Hg should have preventive treatment provided this causes no significant disturbing effects. While the author believes that this would be found to be advantageous, few such patients have been treated in our clinic, with the exception of young women referred to my colleague McQueen by the Department of Obstetrics. Moderate hypertension in these women, liable to further pregnancies, has usually been controlled easily without appreciable interference with normal living.

Improved activity and relief of such manifestations as heart failure, breathlessness, headaches, and giddiness may be obtained in hypertensive patients over the age of 70, and we have found the treatment of such patients has been worth while from their standpoint. Confirmation of this practice comes from the results of Beem.[2] Care must be taken to avoid hypotension, which would carry the risk of fainting and falling, with resultant fractures.

Extent to which blood pressure reduction is desirable

Comparison with our results can only be made if blood pressure reduction corresponds to that obtained in our clinic. While degrees of blood pressure reduction which we would regard as insufficient probably induce some improvement in prognosis, we would emphasize that by adjusting the regimen to the individual, good blood pressure reduction without appreciable discomfort from side effects can be obtained in the great majority of patients. Such results are

Table I. *Eight year mortality rates in untreated patients with hypertension*

Basal blood pressures	*Mortality above*	*Mortality below*
Men		
Systolic 160 mm. Hg	27/28	31/53
Diastolic 100 mm. Hg	26/29	32/52
Women		
Systolic 180 mm. Hg	23/30	31/82
Diastolic 115 mm. Hg	17/22	37/90

usually achieved more easily in grade 2 than in grade 3 patients. There is evidence that patients with adequate blood pressure reduction have a better outlook than those whose reduction is inadequate.[11] Unfortunately, although blood pressure control in some patients is now comparatively simple, in others it requires specialized knowledge, technical assistance, and pertinacious application to the problem over a period of several months or even years. Since I hold the view that high basal blood pressure is harmful in proportion to the degree of its elevation, I am unable to agree with those who, while recognizing the importance of reducing the blood pressure, condone or even recommend procedures which quite clearly do not maintain the blood pressure at near-normal levels during a 24 hour day. For example, many authors are prepared to have the patients lying flat or nearly flat in bed at night, even when the patients are on ganglion-blocking or sympatholytic drugs. If hypertension as such is responsible for the disabilities associated with hypertensive disease, the aim of management should be to maintain the blood pressure at as near a normal level as is practicable. When a drug depends on postural hypotension for its effect, an adequate fall of blood pressure is not obtained unless the patient is sufficiently tilted. A gravitational decrease of the local endarterial pressure in the cerebral arteries is probably also advantageous.

The response to the drugs given will determine the extent to which blood pressure is reduced in a particular case. Some patients who, no doubt, have obstructions in one or more cerebral arteries may develop a temporary unilateral paresis if the blood pressure falls to normal or below. The paresis should disappear within an hour or less if the patient who has received a ganglion-blocking drug lies flat or the blood pressure is raised promptly in some other way. Some patients with coronary disease have less angina if the blood pressure is reduced a moderate degree, but hypotension may cause spontaneous attacks of anginal pain.[7] If hypotension is allowed to persist in these patients, it may lead to electrocardiographic changes characteristic of coronary insufficiency or, rarely, to a frank cardiac infarction. Whereas in most patients we try to reduce the blood pressure to as near normal as practical, in patients with manifest cerebral vascular or coronary artery disease we limit the degree of blood pressure reduction to avoid symptoms such as unilateral weakness or increase of anginal pain, adjusting the degree of blood pressure reduction to the individual. It is rarely necessary to abandon hypotensive treatment, because in most patients reactions can be avoided by attention to the extent of blood pressure reduction. One may have to be satisfied with a modest fall when adequate reduction of blood pressure is obtained only in association with substantial side effects. In the majority of such patients a comfortable and adequate regimen can be constructed, although it may involve many clinical trials.

Choice of drugs for use in hypotensive therapy

In severe cases with advanced retinal changes, hypertensive heart failure, or cardiac asthma, the blood pressure is seldom brought under control without the use of ganglion-blocking drugs. However, where no urgency is apparent, diuretics, alone or with Rauwolfia alkaloids, may suffice. If the basal blood pressure is high, more powerful drugs are needed; time is

saved even in nonurgent cases by use of these from the outset. The hypotensive drugs available in 1960 may be subdivided into two main categories:

A. Mild drugs suitable for background therapy. These are the hypotensive diuretics—chlorothiazide and hydrochlorothiazide—and the Rauwolfia alkaloids—reserpine, rescinnamine, deserpedine (canescine), and various alkaloidal mixtures.

B. The powerful gravity-augmented hypotensives. All reduce the blood pressure by decreasing the activity of the sympathetic nervous system,[27] thereby causing decrease or loss of postural control over the circulatory system. Hence, the fall in blood pressure is greatest while the patient is standing, of intermediate degree sitting, and least lying. All the powerful hypotensive drugs at present available fall into this category. Five different chemical groupings are involved. The ganglion-blocking drugs such as hexamethonium, pentolinium (Ansolysen), mecamylamine (Inversine), and pempidine (Perolysen) which block both the parasympathetic and sympathetic nervous systems may be quaternary ammonium compounds, secondary amines, or tertiary amines. The sympatholytic members of this group (drugs without effect on the parasympathetic system) are bretylium tosylate (Darenthin), a quaternary ammonium compound, and possibly guanethidine (Ismelin), which is an amidine. An analysis of the action of guanethidine shows that the falls of blood pressure which it induces are less influenced by posture than are the falls of blood pressure from any of the ganglion-blocking drugs or bretylium tosylate. This raises the question of whether it acts centrally or on a different site in the sympathetic system.

Treatment by combination of drugs. Before bretylium tosylate and guanethidine became available, the combined use of Rauwolfia alkaloids and ganglion-blocking agents made it possible to have effective blood pressure reduction, without important side effects, in a majority of patients. Further improvements have been obtained by the use of bretylium tosylate and guanethidine alone, in combination with background therapy, or with the addition of ganglion-blocking drugs. Certainly, the most satisfactory regimens in patients with severe hypertension are obtained by the use of drugs in combination.

Background

Rauwolfia alkaloids. Although Rauwolfia alkaloids had long been used in Indian medicine, their properties were unrecognized by the Western world until publication by Vakil.[34]

It is generally considered that reserpine decreases the activity of the central connections of the sympathetic nervous system in the hypothalamus by some means related to the release of 5-hydroxytryptamine from nervous tissue in that region.[3] A peripheral action was demonstrated by McQueen, Doyle, and Smirk,[14] using a rabbit in which the circulation of the hind limbs was separated from the rest of the circulation by means of cross-perfusion. After injections of reserpine into the upper half of the circulatory system, a fall in blood pressure was associated with a rise in perfusion pressure of the vascularly isolated but normally innervated hind limb. A decrease in perfusion pressure occurred, however, when reserpine was added directly to the circulatory system of the normally innervated but vascularly isolated hind limb.

The observations of Burn and Rand[5] that reserpine causes a dispersal of noradrenaline from the region of the sympathetic nerve endings may afford an explanation for the peripheral action of reserpine.

Reserpine (Serpasil) is readily available, but two other alkaloids—deserpedine and rescinnamine—with similar modes of action are of appreciable value, although not readily available.[26, 27] Reserpine is the Rauwolfia alkaloid mainly employed; the total daily dose should ordinarily be 0.25 mg. and should not exceed 0.5 mg. daily. Exceptionally, blood pressure falls to near

normal may be obtained with Rauwolfia alkaloids only. Given by themselves, they are insufficient, but in almost all cases they potentiate the action of the more powerful gravity-augmented hypotensive drugs, so reducing the requisite dose and decreasing any side effects. Some augmentation of the action of ganglion-blocking drugs may at times be obtained even with 0.125 mg. daily.

Side effects of Rauwolfia alkaloids. Doses of reserpine in excess of 0.5 mg. daily (we used 1.5 mg.) were found to produce severe mental depression or lead to suicide in some patients.[8, 10] Mental depression occurs occasionally with 0.5 mg. daily, seldom with 0.25 mg., but with few exceptions the symptom clears rapidly on cessation of administration if only small doses have been used. Sensations of lassitude, blockage of the nose because of congestion of the mucosa, and nightmares are well-known side effects. Occasionally, there are recurrences of the symptoms of peptic ulcer, biliary disease, or bronchial asthma probably caused by unbalanced sympathetic inhibition. Equivalent hypotensive doses of rescinnamine or deserpedine sometimes avoid side effects which have been troublesome with reserpine. The effects on the mind are by no means always unfavorable, and decreases of nervous tension may occur.

Hypotensive diuretics. The drugs of this group at present available include chlorothiazide,[35] usually administered in a dose of 0.5 Gm. night and morning, and hydrochlorothiazides,[9, 17] usual dose 50 mg. night and morning. All these drugs lead to some loss of potassium, and we advocate the administration of 300 mg. of potassium chloride in a sugar-coated pill three times daily.

When administered alone in the above-mentioned doses, some reduction of the blood pressure is usual, and occasionally, patients with severe hypertension show remarkable decreases of blood pressure even to near-normal levels. In general, however, severely hypertensive patients are not adequately controlled with these drugs alone, but almost always, if not invariably, they enhance the response to the gravity-augmented hypotensives and in this way reduce their requisite dose and lessen side effects. This appears to apply to all of the gravity-augmented hypotensives irrespective of their chemical nature or their pharmacologic properties.

The relationship between diuretic action and hypotensive activity is of considerable interest. Enhancement of the action of ganglion-blocking drugs during mercurial diuresis had been noted in our clinic before chlorothiazide became available.[27] My colleagues McQueen and Morrison made a particular study of this. They found that doses of chlorothiazide, hydrochlorothiazide, and mersalyl in approximately equipotent diuretic doses had about the same effect on the blood pressure. A specific hypotensive action unassociated with salt and water metabolism has not as yet been demonstrated with these drugs. McQueen and Morrison* found evidence that their effect was not solely the result of augmented sodium excretion. They discovered a close relationship between the effect on blood pressure and the effect on extracellular volume. I have noted in patients in whom heart failure was not completely relieved by blood pressure reduction alone that the removal of accumulated fluid, after digitalis administration, but without diuretics, was repeatedly accompanied by a decrease in the amount of ganglion-blocking drug required. Clearly there is an important relationship between extracellular fluid and blood pressure.

Side effects of chlorothiazide and hydrochlorothiazide. Side effects were noted in 19.4 per cent of our 170 patients receiving chlorothiazide and in 13.1 per cent of 61 on hydrochlorothiazide. The untoward effects were milder in the patients on hydrochlorothiazide. The main symptoms were nausea and epigastric discomfort, dysuria, skin

*E. G. McQueen and R. B. I. Morrison: Unpublished data, 1960.

irritation, and lethargy. Loss of potassium may give rise to fatigue and other symptoms. In patients who are on digitalis, potassium loss may induce digitalis toxicity. We had such a case in our series.

Combination of a Rauwolfia alkaloid and a hypotensive diuretic. Without doubt, the combination of hydrochlorothiazide and reserpine may induce a useful fall of blood pressure when neither drug alone does this. We have also found no objection to the use of these two classes of drugs in combination as a form of background therapy.

Gravity-augmented hypotensive drugs. From a practical standpoint, the drugs which depend in part for their actions upon the induction of postural hypotension may be subdivided into two main therapeutic groups: First there are the ganglion-blocking drugs[1, 16, 18] which block both the sympathetic and parasympathetic systems. The best known examples are hexamethonium, pentolinium (Ansolysen), chlorisondamine (Ecolid), trimethidinium (Ostensin, Camphidonium), mecamylamine (Inversine), and pempidine (Perolysen). Pempidine and two of its homologues are the most potent of the ganglion-blocking drugs at present available. The two homologues on clinical trial are M & B 5409 and M & B 4500,[30, 31] the latter having approximately twice the potency of pempidine. The second group of drugs acts only on the sympathetic nervous system, so that no parasympathetic side effects occur. Available at present are bretylium tosylate[4, 12] and guanethidine.[15] Their mode of action is less well defined than that of the ganglion blockers. Bretylium tosylate appears to inhibit sympathetic activity in the region of the nerve endings and to some extent probably inhibits conduction down nerve trunks, although it is uncertain whether this latter action occurs under therapeutic conditions.

In practice, the important distinction between the ganglion-blocking and the sympatholytic drugs is the absence in the latter of parasympathetic side effects. Their effect can be explained by reduction in sympathetic activity. Many of the actions held in common by these powerful gravity-augmented hypotensives are explicable by their interference with the normal operation of homeostatic circulatory controls.

Guanethidine, however, is of particular interest pharmacologically because postural hypotension with it is less pronounced than with ganglion-blocking drugs and bretylium tosylate. The blood pressure reduction is slower in onset and may last for several days after withdrawal of the drug.

Much work has been done on the pharmacologic nature of hexamethonium and, although other ganglion-blocking drugs and bretylium tosylate have not been studied in equal detail, it seems likely that most of the changes resulting from interference with homeostasis through the various actions on the sympathetic nervous system will be found to apply to all of them. In all these drugs the fall of blood pressure is greatest in the standing posture and is sometimes slight when the patient is lying. The degree to which the blood pressure falls during drug action on assuming the standing posture is mainly an individual characteristic but, as Dern[6] showed, there are individual differences between ganglion blockers. The blood pressure fall after guanethidine also is influenced by posture, although less so than with ganglion blockers and bretylium tosylate. Reactions which are characteristic of the ganglion-blocking action of hexamethonium will probably be found to apply equally to other ganglion blockers, to bretylium tosylate, and possibly to guanethidine. The blood pressure fall is enhanced by all stimuli which induce increased activity of the sympathetic nervous system, namely, salt loss, blood loss, fluid loss by strong purgation, diarrhea, and strong diuretics, fever, and surgical injury. The effect of these drugs on the blood pressure is diminished by homeostatic stimuli leading to decrease of sympathetic discharge, namely, blood transfusion, infusion of dextran (even the salt-free form), and external pressure on the body surface.

The parasympathetic side effects are common to all ganglion-blocking drugs, although the emphasis on the various individual unpleasant parasympathetic effects varies from one individual to another and from one drug to another.

Choice of a gravity-augmented hypotensive drug. All the ganglion-blocking and sympatholytic drugs mentioned above are effective hypotensive agents. Some of the ganglion-blocking drugs such as pentolinium and chlorisondamine have the advantage of a long record of freedom from delayed toxicity, and where a comfortable regimen has been established, there is no advantage in changing. If a patient is not already being treated with one of these substances, convenience will prompt many physicians to make a first choice of some other drug because with pentolinium and chlorisondamine, drug tolerance delays the development of a relatively stable regimen. For some time, however, these substances have been most useful, and for a number of patients they will remain the most satisfactory of the ganglion-blocking hypotensive agents.

It is well to state at this point that there are individual reactions to all gravity-augmented drugs such that patient A is clearly more comfortable on drug Y than on drug X, whereas patient B is more comfortable on drug X than on drug Y. The differences in individual reactions to these drugs are not merely the expression of irrational preferences.

TRIMETHIDINIUM (OSTENSIN). This drug is of interest in that it resembles pentolinium but does not lead to significant tolerance. In some patients on trimethidinium, visual blurring is more prominent than other unpleasant parasympathetic effects. Mecamylamine and pempidine are amines and have an advantage in potency over earlier drugs, partly because of the complete absorption of oral doses. Neither leads to significant tolerance. All the usual undesirable parasympathetic effects occur with both mecamylamine and pempidine. It is my impression that pempidine is preferable, because mecamylamine sometimes causes delayed toxicity characterized by gross tremors, excitability, and even psychotic manifestations. We have observed no such delayed toxicity with pempidine or with two of its homologues (M & B 4500 and M & B 5409A) to which we have given an extensive clinical trial.[30, 31] When a ganglion-blocking drug is required, our first choice at present therefore is pempidine or one of its homologues.

Although our experience with sympatholytic drugs is necessarily of shorter duration, there is much to be said for using them as alternatives to ganglion-blocking drugs when blood pressure reduction is not urgent. They are less suitable than ganglion-blocking drugs for the expeditious introduction of a hypotensive regimen, since it is not always possible to obtain sufficient blood pressure reduction with them.

BRETYLIUM TOSYLATE. A quaternary ammonium compound, this drug was the first to be introduced by Boura and associates[4] and is a most important therapeutic agent. Mild cases may be controlled by doses three times daily of bretylium tosylate without adjuvants, but it is often preferable to administer bretylium tosylate in combination with background therapy of hydrochlorothiazide or a Rauwolfia alkaloid or a combination of these. Used alone or in combination with other hypotensive drugs it has increased the proportion of patients in whom effective control over the blood pressure can be obtained with no really troublesome side effects. In a proportion of patients, the dose of bretylium tosylate, even in combination with background therapy, rises to 600 mg. or higher three times daily and becomes associated with indigestion or some unpredictability of hypotensive action. Facial pain over the parotid gland, when eating, is a peculiar and not infrequent side effect of this drug. Even after doses of 600 mg. three times daily, blood pressure reduction may be inadequate in some severely hypertensive patients. In such patients we find that the

Table II

Drug (oral)	*Initial single dose (mg.)*	*Increment by which dose may be raised or lowered (mg.)*
Pentolinium (Ansolysen)	20	20 (occasionally 10)
Chlorisondamine (Ecolid)	12.5	12.5
Trimethidinium (Ostensin)	20	20
Mecamylamine (Inversine)	1.25-2.5	1.25-2.5
Pempidine (Perolysen)	1.25-2.5	1.25-2.5
Bretylium tosylate (Darenthin)	100	50-100
Guanethidine (Ismelin)	10	5-10

The increments of doses are adjusted to size of tablets available commercially.

addition of a highly potent ganglion blocker, preferably pempidine or one of its homologues, can be used to replace some of the bretylium tosylate or to increase the hypotensive action of the regimen to a sufficient degree.[12, 30] We have performed numerous trials of a combination of bretylium tosylate and a potent ganglion blocker. It is quite clear that in suitable combinations the amount of bretylium tosylate administered can be greatly reduced without return of the parasympathetic manifestations of ganglionic blockade.

Approximately equivalent hypotensive activity results from 100 mg. of bretylium tosylate, 2 to 4 mg. of pempidine, 2 to 4.5 mg. of M & B 5409A, or 1 to 2 mg. of M & B 4500. Although no such preparation appears to be available at present, the combination in one tablet of suitable proportions of bretylium tosylate and a potent ganglion-blocking drug would have advantages.

GUANETHIDINE. In dose of 60 to 180 mg. daily, guanethidine may not reduce the blood pressure for several days, but the effect is cumulative and a dose which on the first day of administration has little effect on the blood pressure may prove to have excessive action when given daily. When the blood pressure is reduced with guanethidine, after withdrawal of the drug the return to the original level may take several days. The side effects encountered have been general malaise, sensations of nervous tension, diarrhea, and general muscle pains. This agent deserves an extended trial, but it is unsuitable for urgent cases because of uncertainty concerning the extent to which blood pressure reduction is feasible.

Practical detail of hypotensive therapy. Probably the best form of background therapy at the present time is hydrochlorothiazide, 50 mg. night and morning, with 300 mg. of potassium chloride three times daily to replace potassium loss in the urine. Undesirable effects have been much less frequent with hydrochlorothiazide than with chlorothiazide.

Reserpine, preferably 0.25 mg. daily, but sometimes 0.5 mg. daily, may be used as an alternative to or in addition to hydrochlorothiazide. Undesirable effects may be entirely absent at this dose level, but when present they may develop insidiously and may be unrecognized by the patient.

The method we have used for regulating the dosage of all ganglion-blocking drugs applies to bretylium tosylate but not to guanethidine (Ismelin). To obtain the best results in the more severely hypertensive patient requires detailed knowledge, a discussion of which is outside the scope of this article but may be obtained elsewhere.[27, 32]

1. The requisite dose of ganglion-blocking drugs and of bretylium tosylate is highly individual and has to be discovered by starting with a small dose and working up by small standard increments until an effective dosage regimen has been developed. Table II sets out suitable initial doses and increments. If a dose proves to be too large, small reductions are usually appropriate. Large dose reductions commonly decrease it to below the threshold of effectiveness.

2. In general, the object is to reduce the reading in the trough of the blood pressure fall to normal, pressures being measured in

the standing posture. If the blood pressure reduction at the trough of the curve is insufficient, the duration of the fall in pressure is likely to be brief.

3. Because the degree of blood pressure reduction is usually slight with lying posture, patients on ganglion-blocking drugs are advised to sleep propped up in bed with a back rest at 45 degrees or to have the head of the bed raised on blocks 16 inches high.

4. Casual blood pressure determinations at an outpatient clinic are often deceptive. All-day tests are a valuable check. When the patient complains of hypotensive symptoms but has a high casual blood pressure at an outpatient visit, it is likely that at other times the blood pressure falls to a low level. The method of using symptomatology as a guide to the adequacy of a hypotensive regimen has been described elsewhere.[25, 27]

5. In the case of guanethidine, experience is comparatively limited. The effective dose is usually of the order of 20 to 40 mg. three times daily, and on such a dose several days may elapse before the full effect on the blood pressure becomes apparent. Some patients have an excellent fall of blood pressure with no side effects; others do so for a time and then develop side effects and wish to stop the drug.

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Erratum

Through oversight, the quantity of insulin employed in the experiments reported was not mentioned in the article, "Effect of Epinephrine, Insulin, and Tolbutamide on Carbohydrate Metabolism During Ether Anesthesia," by Dorothy H. Henneman and Leroy D. Vandam (CLIN. PHARMACOL. & THERAP. 1:694-702, 1960). The quantity injected intravenously was 0.1 U. of regular insulin per kilogram.

Symposium on the experimental pharmacology and clinical use of antimetabolites

Part V. Use of combinations of antimetabolites for chemotherapy of cancer

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It is possible to view the approach to a chemotherapy of cancer more rationally if it is given a frame derived from a recent concept of how cancer arises and progresses. This concept will be referred to as the "deletion concept." The experimental evidence for this concept is available mainly from studies on chemical carcinogenesis and has been discussed elsewhere.[16, 20-22] By this concept, a cancer cell arises when a normal call is subject to deletions that are not lethal, but leave it without the components responsive to normal growth controls. What specific deletions are necessary to achieve this result are as yet unknown. It may be that the same net result can be reached by any of several different deletions. Potter[21] has suggested one plausible way in which this could occur and cites several examples. When cells have alternate metabolic pathways such that a substrate can be either catabolized for energy or used to synthesize new material for cell growth, the deletion of the catabolic pathway could lead to uncontrolled growth. One example cited is that of uracil, which can be used by liver cells to synthesize uracil nucleotide—a precursor for nucleic acid synthesis—or oxidized to carbon dioxide, water, and ammonia. Liver cells have powerful catabolic systems and use uracil for the synthetic pathway only when it is available in large amounts. In intestine, a growing normal tissue, and in hepatomas the catabolic pathway is weak. The result is that the latter two tissues readily use uracil for nucleic acid synthesis.

The "deletion concept" provides a common ground for the varied views currently prevalent concerning carcinogenesis. The cell has been shown, by isotopic tracer experiments, to be in a very dynamic state. All the components are constantly being degraded and resynthesized, except the chromosomal material, which is also dynamic during cell reproduction. Any factor which slows or competes with the duplication of a cell component may cause its deletion. There are probably many lethal deletions for every carcinogenic deletion. Chemicals could lead to deletion by combining with and eliminating cell components from the active system. Viruses may complete for nutrients with cell components, or inhibit their reproduction, or replace some of the chromosomal material of the cell. Hormones certainly influence the

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rates of synthesis of cell components and may cause such imbalances in rates as to result in deletions.

One would expect that deletions of metabolic equipment not essential to growth would continue in the cancer cells, and such "progression" is certainly observed. Tumors become more anaplastic and converge to simpler metabolic patterns. Primary adenocarcinomas of the breast may initially be hormone dependent and progress to hormone-independent tumors. Thyroid carcinomas may or may not produce thyroid hormone, as did their cells of origin. This progression toward a common metabolic pattern was discussed by Greenstein.[7] However, the various differentiated tissues have widely differing metabolic equipment, developed for the performance of their specialized functions. How much of this is still present in the primary tumors would seem to be largely a matter of chance. The observations of Greenstein concerning convergence of tumors have more recently been found to be somewhat misleading by the workers concerned with studies of cancer chemotherapy. Even though much of the specialized metabolic equipment of a cell may have been deleted along with that lost in the deletion which was carcinogenic, the fragments of this equipment left can lead to greatly varied responses to drugs used for chemotherapy. The result, as this author[15] pointed out earlier, is that chemotherapy must be able to treat cancer as a large group of diseases. Any one drug treatment will probably affect only a small percentage of a spectrum of clinical cancers. In order to operate an effective cancer chemotherapy, it will be necessary to have a series of cancer-inhibiting drugs, to determine the mechanism of action of each, to study the mechanisms of resistance to the drugs, and to have biologic test systems which permit determination of the drugs to be used in each specific cancer case.

As Potter[20] has pointed out, substrates for cellular metabolism can proceed through "alternative metabolic pathways," and it is quite usual for cells to have several ways of accomplishing the synthesis of a required component. This is especially true for the tumor cell, which may obtain some components totally or partially synthesized by host cells. As a consequence of this, it is likely that combinations of antimetabolites will be required to accomplish successful arrest of tumor growth in at least some cases.

One of the most pressing problems in studies of cancer chemotherapy has been and is the emergence of populations of cells which are resistant to the drugs used. Quite dramatic responses have been obtained in clinical cancers, particularly the acute leukemias, with some presently available drugs such as 6-mercaptopurine and amethopterin. However, these responses are soon lost as a result of the development of drug resistance. This will not be discussed in any detail here, since it is the subject of another paper in this symposium. It should be mentioned, however, that the solution to this problem probably also lies in the use of combinations of drugs to meet the use of alternate pathways of metabolism, which are in some cases the means by which cells continue to grow in the presence of the drugs.

As an experimental approach to test and, if possible, illustrate the solution to the concepts just discussed, we have selected antimetabolites for purine metabolism. These have been studied in some detail and seem to illustrate the principles outlined above. Our experimental tools were a series of ascites cell tumors of mice, some of them in inbred mouse lines. These included examples from some of the major tumor types, mammary adenocarcinoma, sarcoma, lymphosarcoma, and included tumors responsive and unresponsive to the drugs used. The first drug studied in our program was azaserine, an antibiotic discovered in a Streptomyces culture filtrate and reported to have inhibitory activity for Sarcoma 180 in mice.[28] It was soon shown by Hartman and co-workers[8] to be an inhibitor of a glutamine reaction in the se-

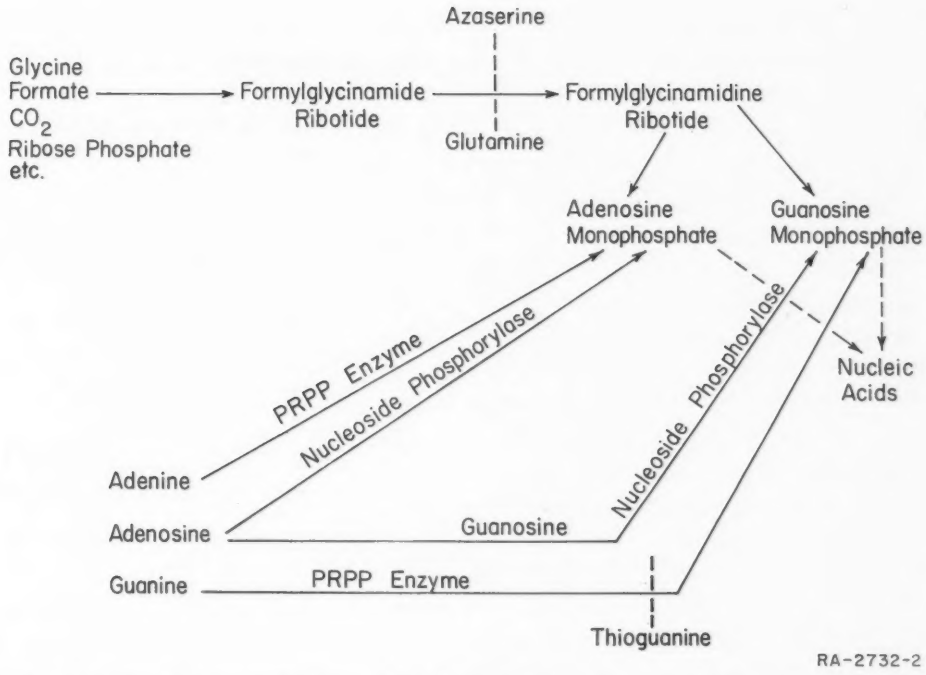

Fig. 1. Diagrammatic representation of the interreactions in purine nucleotide synthesis.

quences of enzymatic steps leading to the synthesis of purines by preparations of pigeon liver. This site of inhibition is illustrated in Fig. 1. Buchanan[3] then assumed azaserine to be a competitive antagonist of glutamine. However, our experiments[6, 14] with ascites cell mouse tumors, in vivo and in vitro, showed that azaserine reacted irreversibly with a cell constituent. The cells could not be washed free of the azaserine, and glutamine in relatively large amounts could partially prevent but not reverse the inhibitory effects of azaserine.[6] An illustration of this is provided in Fig. 2, where it can be seen that azaserine produced a complete block of purine synthesis after the first few minutes. This indicated that azaserine was combining with the enzyme concerned in the reaction. Just as was the case in the pigeon liver extracts of Hartman,[8] these ascites cells were found to accumulate relatively large amounts of α-N-formyl glycinamide ribotide in vivo under the influence of azaserine.[17]

When mice bearing ascites cell tumors or solid tumors were given intraperitoneal doses of azaserine, in vivo inhibition of *de novo* purine synthesis by the tumor cells could be shown to be almost complete for approximately 12 hours. After this time the inhibition fell off sharply, probably indicating resynthesis of enzyme. This is shown in Fig. 3 for subcutaneous growths of Ehrlich carcinoma. It thus seemed likely that treatment should be at 12 hour intervals to be most effective. Data presented in Table I illustrate the results obtained when groups of mice bearing various ascites cell tumors were treated for 6 days either once or twice a day (with the same total dose) and survival times determined. It is evident that a much better response is achieved with dosage given twice a day, in correlation with the biochemical studies.

In an attempt to determine the relative sensitivities of normal and neoplastic mouse tissues to azaserine, Moore and LePage[17] administered various doses of azaserine to tumor-bearing mice and, by radioactive tracer measurements, determined the extent of inhibition of purine synthesis in various tissues at each dose level. A plot

of the results showed a break in the curves at approximately 90 per cent. The dose producing this level of inhibition in a variety of mouse tissues has been given in Table II. It is evident that the tumor cells are the most sensitive of those tested, especially when they have direct contact with the drug, as is the case with the ascites form. The spleen, which increases several fold in size in these tumor-bearing mice and therefore is a growing tissue, is the most sensitive of the normal tissues tested. The great resistance of the liver to this drug can be explained, as it has a high level of an enzyme which destroys the drug by deamination. Here is an example of how a normal tissue can be protected from a drug. In the deletion process, any tumor which arises in a tissue possessing such a protective enzyme would have the potentiality of resistance by reason of retaining this enzyme. However, those tumors which suffered deletion of this enzyme, or originated in differentiated tissues not possessing the enzyme, would potentially be sensitive to the drug.

An observation that may have interest in regard to the dosage schedule for use of azaserine was made in these studies. Ellison and his associates[5] observed in the clinical trials of azaserine that patients given doses of 10 to 20 mg. per kilogram of body weight developed a pronounced stomatitis, which became sufficiently serious to require cessation of treatment. No such "side effect" was observed in mice. Once we knew that azaserine was "titrating" an enzyme in the cancer tissue and producing an irreversible inhibition, one could see that the situation most likely to give the best therapeutic index for the drug would be that in which it was present in the blood only long enough for reaction with the enzyme component of the tumor. To have it present much longer would result only in successive "titration" of the less susceptible normal cells. It then seemed possible that the "side effects" obtained in humans, but not in mice, were a result of longer retention of the drug in human blood and "titration" of the relatively sensitive gastric mucosa. This was studied by Henderson, LePage, and McIver,[11] who assayed blood sera from mice and humans receiving various doses of azaserine. Data presented in Fig. 4 illustrate that azaserine is indeed eliminated from mouse blood within a few minutes, but that relatively small doses cause prolonged presence of the drug in human sera. The larger doses

Table I. *Effect of azaserine on the average survival time (days) of mice bearing ascites cell tumors*

Tumor	Controls	Azaserine treated*	
		Once daily	Twice daily
Ehrlich (carcinoma)	10	13	19
TA3 (mammary adenocarcinoma)	8	11	15
Sarcoma 180	8	13	18
6C3HED (lymphosarcoma)	13	17	22

*Treatment was started 24 hours after transplanting and continued at 0.4 mg. per kilogram per day for 6 days.

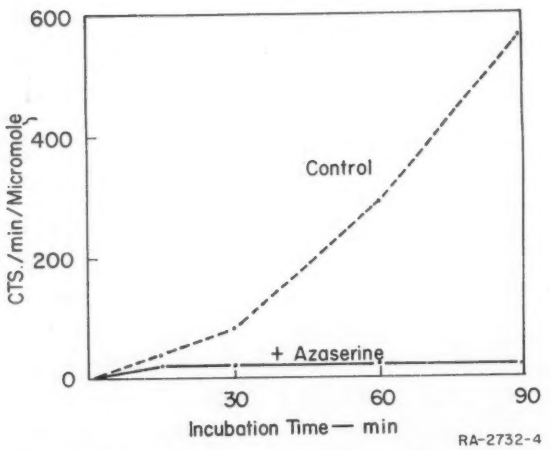

Fig. 2. Rate of *de novo* synthesis of purines in Ehrlich ascites cells, in vitro, as measured by glycine-2-C^{14} incorporation. Reaction vessels were incubated at 38° C. with 120 mg. of ascites cells in 12 ml. of a salts-glucose medium buffered with bicarbonates, containing 150 μg glucine-2-C^{14} with or without 0.1 μg azaserine. (From Greenlees and LePage.[6])

resulted in longer retention of azaserine in the blood. This would support the premise that azaserine should be given to humans only in much smaller doses than were used in early trials,[5] and that these smaller doses can give a sufficiently prolonged blood level. While it does not so prove, this observation strongly suggests that the toxicity observed in humans results from prolonged presence of azaserine in the blood such that "titration" of enzymes concerned with purine synthesis in the gastrointestinal mucosa occurs.

It is interesting to note that, while the development of transplanted mouse tumor lines resistant to purine analogues such as 6-mercaptopurine has been relatively easily accomplished, Sartorelli and LePage[23] were able to obtain an azaserine-resistant line of tumor cells only after several attempts, and even then some thirty transplantation generations in treated mice were necessary. The study of this drug-resistant line of cells supported the concept that the inhibition of purine synthesis was relieved only after a new component of enzyme was synthesized. Both sensitive and resistant lines were inhibited by the drug, but the recovery of synthetic capacity at a more rapid rate was the basis for resistance in the resistant line.

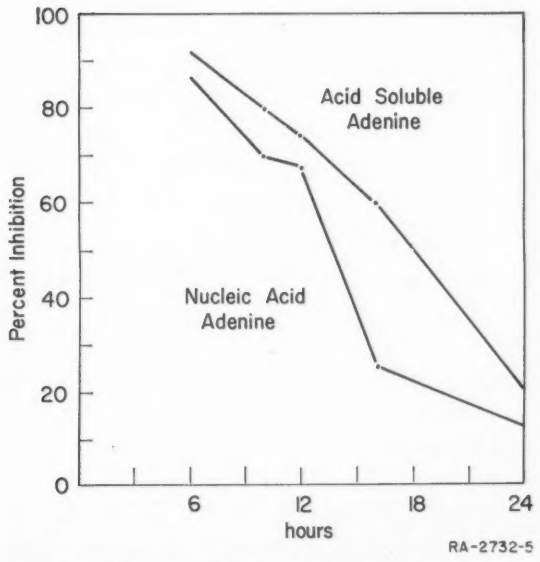

Fig. 3. Duration of the inhibition of *de novo* purine synthesis in solid Ehrlich carcinoma implants after a single 6 mg. per kilogram dose of azaserine. Synthesis was measured by administering glycine-2-C^{14} at the indicated time and sacrificing the mice 1 hour later for isolation of purines from the tumors. (From Henderson, LePage, and McIver.[11])

Table II. *Doses of azaserine found to produce 90 per cent inhibition of purine synthesis in mouse tissues*

Tissue	*Dose (mg./kg.)*
Ehrlich carcinoma ascites	0.2
Ehrlich carcinoma (solid)	2.9
Spleen	7.2
Intestine	14
Kidney	57
Lung	110
Liver	180

From Moore and LePage.[17]

It became evident that azaserine was unable to produce complete inhibition of growth in experimental cancers because they could either synthesize purines or obtain them from the host.[15] Evidence since published by Henderson and LePage[9, 10] showed that all the experimental tumors examined could utilize host purines at appreciable rates. Azaserine did not inhibit this process.[15] Obviously a combination of chemotherapeutic agents was indicated, and an inhibitor of the utilization of host purines was sought. We were guided by available information concerning the character of purine metabolism in such rapidly growing normal tissues as intestinal mucosa and bone marrow. In the former, rapid *de novo* purine synthesis is observed. In the latter, however, there is little, if any, capacity for purine synthesis[1] and evidence is available that bone marrow depends upon the liver for purine components.[12] Biochemical studies of 6-mercaptopurine[6] indicated that it was an inhibitor of some purine coenzyme function rather than a purine nucleotide synthesis for nucleic acid replication. The 6-mercaptopurine is inhibitory to both intestinal mucosa and bone marrow.

In contrast, 6-thioguanine was reported by Clarke and collaborators[4] and by Philips and his associates[19] to be specifically inhibitory to the bone marrow. We were thus led to test thioguanine in combination with azaserine. As reported by Sartorelli and LePage,[24] this combination of chemicals gave a synergistic response when used to treat certain of the experimental mouse tumors. Treatment of mice bearing Sarcoma 180 or Ehrlich carcinoma for only 6 days with this combination resulted in 50 to 60 per cent of complete remissions. It was demonstrated that simultaneous administration of the two drugs was necessary to achieve this result. With TA3 adenocarcinoma in compatible mice, 23 per cent of the mice showed complete remission. Mice from the latter experiment, without detectable tumors, were challenged 50 days later with the same tumor and left untreated. They had the same survival time as controls, indicating that no immune response was involved in the remissions.*

While this work was in progress, a report by Tarnowski and Stock[29] appeared, reporting the use of various combinations of drugs in the treatment of two mammary carcinomas of mice, RC and S-790. The former gave a synergistic response to combined treatment with azaserine plus thioguanine. The latter gave very little response to any chemical treatment. Sartorelli and LePage[24] showed that two mouse tumors of their series, Mecca lymphosarcoma and 6C3HED, did not respond at all to the combined treatment with azaserine and thioguanine. They also derived a thioguanine-resistant line of Ehrlich carcinoma cells, which, however, did respond to treatment with azaserine plus thioguanine. The tumor was shown to have capacity to degrade thioguanine by deamination and oxidation to thiouric acid. Here again is an illustration of a way in which tumors may vary in response to a drug as a result of "metabolic equipment" retained from their cells of origin. It also illustrates a way in which one can hope to discriminate between growing normal cells and some tumor cells. Certain of the normal cells, those of intestinal mucosa, for example, possess a guanase which deaminates and thus inactivates thioguanine. They are thus protected from this drug. Those tumors which arise from cells possessing a guanase will also be so protected, but some will have this specialized equipment deleted and will therefore potentially be sensitive to the drug.

Sartorelli and co-workers[25] observed that the derived line of Ehrlich carcinoma cells, which was resistant to thioguanine, did not incorporate appreciable amounts of the drug into cellular nucleic acids. In contrast, the sensitive line of cells showed relatively large incorporation of thioguanine into nucleic acids. When the resistant line was treated with both azaserine and thioguanine, to which it was susceptible, then incorporation into nucleic acids was as high as in the sensitive cell line. Regardless of whether or not nucleic acid incorporation of the drug damages the cells, this presented an attractive possibility as a test of tumors for susceptibility to this chemotherapeutic regimen. Any means by which cells achieved resistance (e.g., impermeability, destruction of drug, inability to form nucleotides, or specificity of enzymes

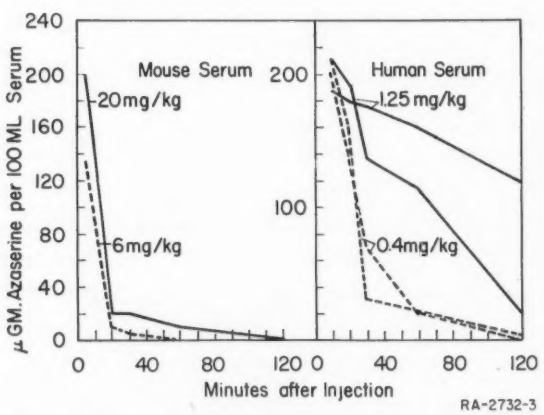

Fig. 4. Rate of elimination of azaserine from the blood serum of mouse and human after administration of an intravenous dose. (From Henderson, LePage, and McIver.[11])

*G. A. LePage: Unpublished work.

for the normal metabolites) would prevent incorporation of thioguanine into nucleic acids. A survey was made of a number of experimental tumors and normal tissues.[13] Five tumors that respond well to this drug treatment all incorporated thioguanine into nucleic acids, as did bone marrow which is susceptible to thioguanine. Three tumors not responsive to the treatment and a resistant normal tissue, intestinal mucosa, did not incorporate appreciable amounts of thioguanine into nucleic acids. Thioguanine-C^{14} was synthesized and used for a simple test system with tissue slices to determine whether nucleic acid incorporation of thioguanine occurred. Since a correlation between this test and drug susceptibility was obtained in experimental cancer, it will be interesting to determine whether this is useful in selecting clinical cancers for treatment, since this was suggested in the introduction as the desirable objective. One preliminary report has been made concerning treatment of clinical cancer with azaserine and thioguanine in combination.[27] Eight of some 19 patients treated were reported as showing a favorable response to the first treatments.

The author has predicted, and the experiments with a spectrum of mouse tumors already seen to lend support, that many tumors will not respond. It becomes of interest then to consider what thioguanine does to the metabolism of cells and how cells may escape the effects of azaserine and thioguanine treatments.

Several effects of thioguanine on the purine metabolism of ascites tumor cells have been noted.[25, 26] Two of these seem to have sufficient importance to explain the tumor-inhibitory effects; one is the "feedback inhibition" of *de novo* purine synthesis observed,* in which accumulation of α-N-formyl glycinamide ribotide under the influence of azaserine is greatly reduced by the presence of thioguanine; the other

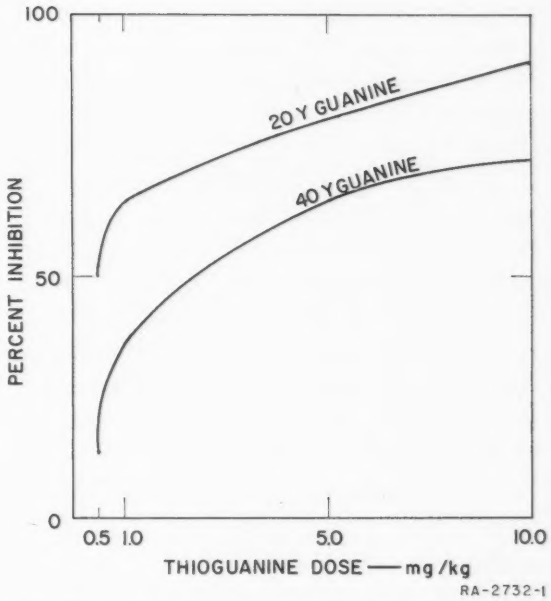

Fig. 5. The influence of varied doses of thioguanine on the incorporation of guanine-8-C^{14} into nucleic acids of Ehrlich carcinoma cells in vivo.

is a competitive inhibition of the utilization of preformed guanine. The first of these has now been largely eliminated as a possibility since it occurred in either susceptible or resistant tumors and was not produced by a tumor-inhibitory derivative of thioguanine.* The latter therefore remains as the most likely site of the important action of thioguanine on cellular metabolism. Illustration of this inhibition of guanine utilization is provided in Fig. 5. The site of the effect is indicated in the metabolic sequences of Fig. 1.

The research on the effects of thioguanine and thioguanine derivatives is being continued with the aim of determining the metabolic basis for resistance in the resistant neoplasms. Brockman and co-workers[2] have made an important finding in regard to tumor cells resistant to purine analogues. They studied a line of mouse Carcinoma 755 which was resistant to 8-azaguanine. The sensitive cell line easily converted the drug to nucleotide form, whereas the resistant line did not. This im-

*"Feedback inhibition" is the inhibition of a metabolic reaction by the products. Pyrimidine synthesis has been shown to be controlled by this means in certain microbial systems.[30]

*G. A. LePage: Unpublished work.

plied that the nucleotide form was the active form in tumor inhibition. It is not as yet known whether resistant tumors encountered in clinical chemotherapy involve this mechanism. If it is assumed that this is the case, then one possible means of circumventing it would be use of the nucleoside form of the drug. It has not been illustrated with experimental material, but the deletion of the enzyme necessary for formation of nucleotide from a free base form of antipurine (PRPP enzyme) would be unlikely to be accompanied by deletion of the enzyme necessary to convert the nucleoside form of the drug to nucleotide (nucleoside phosphorylase). Thus, if the analogy to thioguanine derivatives can be assumed, thioguanine might be useless in a tumor because that tumor lacked a PRPP enzyme, but respond to thioguanosine because it did possess the nucleoside phosphorylase. Nucleotide forms would not be expected to penetrate cells, and thus intracellular synthesis is assumed to be essential.

The thioguanine-resistant tumors in our own series do not respond to thioguanosine. It is merely speculation, but, as can be observed in Fig. 1, cells can interconvert adenine and guanine. This may occur at nucleoside level. It is worth noting that thioguanine has no influence on adenine utilization. There is the possibility, not inconsistent with our data thus far, that the tumors (e.g., Mecca lymphosarcoma, 6C3HED) which do not respond to combined treatment with azaserine and thioguanine are those which can interconvert adenine and guanine sufficiently rapidly that host adenine can supply the need for both these purines. We are interested in finding an inhibitor of this interconversion.

One impression that the author hopes to convey is that there is great need for intimate cooperation between biochemists and those of the medical profession involved directly in the treatment of clinical cancer. This cooperation has at times taken place, but is all too rare. It must increase if chemotherapy is to make a real contribution to the cancer problem.

The author wishes to thank Dr. H. P. Rusch, Editor, *Cancer Research*, for permission to reproduce several charts herein from earlier publications in that journal.

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(To be continued)

Book reviews

Medical Physiology and Biophysics. (Eighteenth edition of *Howell's Textbook of Physiology*), edited by T. C. Ruch and J. F. Fulton, Philadelphia, 1960, W. B. Saunders Company, 1232 pages. $16.00.

How to review a colossus?

It would be improper for me to attempt to review the material presented in *Medical Physiology and Biophysics* critically; this would require a professional physiologist with very broad training. A collaborative review might be even better. I can state that as a pharmacologist, I found whatever I wanted, hoped to find, or can conceive that I will need and found it clearly and logically presented. However, there are some matters of interest which I may undertake to point out.

Whether it be of insects or of physiology textbooks, metamorphosis is always an interesting as well as a mysterious process. To one brought up on that American classic, *Howell's Textbook of Physiology,* this eighteenth edition, which might be called stage five, comes as something of a shock, for although one expects a book to develop with the times, rarely have two editions of the same book, separated only by five years, differed so much from each other as the present edition does from its predecessor. Surely the changes reflect the lively rate of developments in the discipline, but surely they also reflect differences in points of view and in the specialized interests of the authors and editors. What started out in 1896 as *An American Text-Book of Physiology* "edited" by William H. Howell became in 1905 *A Text-Book of Physiology for Medical Students and Physicians* "written" by William H. Howell. In 1946 stage three, *Howell's Textbook of Physiology,* was "edited" by the late John F. Fulton, and in 1949 and 1955 the sixteenth and the seventeenth editions, entitled *A Textbook of Physiology,* were also "edited" by John F. Fulton. The current, eighteenth edition, *Medical Physiology and Biophysics* is "edited" by Theodore C. Ruch and John F. Fulton.

Though listed as co-editor, Fulton obviously took a back seat in the preparation of this edition. All of Fulton's sections on the central nervous system have been written by others. The works of newcomers have completely replaced David Lloyd's extensive contributions to the previous edition. Many of the contributors to other sections are also new.

But most striking of all is the radical change in orientation. As a consequence, the first major change in the title had to be made. "Biophysics" could hardly be omitted, but why "Medical" was added is not clear from my reading, for there is certainly no more than the usual amount of reference to pathologic processes found in most "normal" physiology textbooks. Dr. Ruch's defense of the addition is strained, for he also points out that the orientation of the book is to present physiology "in depth," which, I presume, means emphasis on the basic and intimate details of physiologic function and which also accounts for the addition of "Biophysics." The occasional reference to a pathologic process when it seems to illuminate a point in normal physiology is precisely the opposite approach to that of textbooks on clinical physiology, in which discussions of normal physiology are used to illuminate questions on pathologic processes. Sometimes, however, neither method was followed in dealing with abnormal material; thus the discussion of cardiac arrhythmias is too cursory to be useful to the student who knows nothing of them or to the physician or worker who knows a great deal, while the statement on cardiac murmurs seemed to serve no purpose at all. Fortunately, "Medical" is only a miniscule part of this fine large volume.

The editor states that the book is designed for a "first course" in physiology. It seems to me that it is aimed at a much higher level and that the only concession to the novice is made in the preface. I, for one, should like to audit the course, first or otherwise, in which this book is thoroughly exploited as a text.

No part of what has been said should be taken as a complaint about the content of the book, about the manner in which the material is presented, about the way it is written, or, for that matter, about the decision to write "in depth." It is a large book and, as claimed, it *is* written in depth, but there is nothing murky about the depths; the book is truly readable. Although I did not intend to review any section of this book critically I cannot refrain, in closing, from stating that I thought that the very first chapter, an entirely new one, on the "Biophysics of the Cell Membrane," by Woodbury, a small masterpiece.

Walter Modell

Annual Review of Biochemistry, vol. 29, edited by J. M. Luck. Palo Alto, Calif., 1960, Annual Reviews, Inc. 786 pages. $7.00.

The prefatory chapter of the 1960 *Annual Review* by the late H. O. L. Fischer describes interesting events of his early years in Germany as the son of Emil Fischer, his subsequent career in Canada, and the many happy and productive years at Berkeley. The next chapter is a generalized survey of the mechanism of enzyme action. Boyer considers the two means whereby enzymic catalysis occurs: by change in the electron densities of particular atoms of reactants and by steric shifts or hindrances which strain the bonds of the reactants. Particular emphasis is placed on transfer reactions, reactants, cofactors, essential sites, and atom sources in the reaction products. Boyer favors the mechanism that is viewed in terms of one key transition state. In a more specific review of proteolytic enzymes, Hartley attempts to classify these enzymes on the basis of mechanism of action, rather than origin or specificity. He considers serine, thiol, acid, and metal proteinases as possible categories. Particular emphasis is placed on recent work on the serine proteinases and the use of model compounds. A new chapter on transferases, dealing with reactions which transfer a part of the donor molecules, except hydrogen or electrons, to the acceptor molecule where neither the donor nor the acceptor is water, considers kinases, adenylyl transferases, substituted phosphoryl residues, and glycosyl and acyl transferases.

The chapters on the chemistry of carbohydrates, complex lipids, amino acids, and peptides are expertly done. Of particular interest is the well-written chapter on the structure of proteins. Udenfriend, Weissbach, and Mitoma thoroughly review amino acid metabolism, considering in detail recent pathways of amino acid biosynthesis and conversions. Chapters on carbohyrate and lipid metabolism are also of especial interest. Sulfur metabolism is reviewed for the first time in 14 years. Clinical chemistry is limited to bile acids in blood and magnesium metabolism. Particular emphasis is placed on oxytocin, vasopressin, and the melanocyte-stimulating hormones in the chapter on the biochemistry of protein hormones.

Chapters on the biochemistry of viruses, genetic factors, cancer, and immunochemistry are uniformly excellent. The chapter on neurochemistry is thorough. Vitamins, nutrition, and gas chromatography are covered, with specific interests of the authors considered. The last chapter of this excellent review is a generalized treatment of biologic oxidations by Klingenberg and Bucher.

Joseph F. Reilly

Books received

Albert, A.: Selective Toxicity, ed. 2, New York, 1960, John Wiley & Sons, Inc. 233 pages. $5.50.

Eastham, R. D.: Biochemical Values in Clinical Medicine, Baltimore, 1960, Williams & Wilkins Company (exclusive U. S. agents). 144 pages. $3.75.

Laurence, D. R., and Moulton, R.: Clinical Pharmacology, London, 1960, J. & A. Churchill, Ltd. 490 pages. $4.50.

Loéve, M.: Probability Theory, ed. 2, Princeton, N. J., 1960, D. Van Nostrand Co., Inc. 685 pages. $14.75.

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Wolstenholme, G. E. W., and O'Connor, C. M., Editors: Congenital Malformations (Ciba Foundation Symposium), Boston, 1960, Little, Brown & Company. 308 pages. $9.00.

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Wuhrmann, F., and Wunderly, C.: The Human Blood Proteins, London, 1960, Grune & Stratton, Inc. 491 pages. $15.75.

Correspondence

[That letter writing is fast dying as an art in this country is merely corroborated by the fact that although a Correspondence Section has been available in this journal since its inception, no one has asked to use it until now. It seems clear enough that since the impersonal telephone not only provides immediacy of communication but also eliminates all problems of sentence structure and syntax, it is a much easier way out for most of us. However, this journal cannot offer a Telephone Department. It is with some special pleasure therefore that we publish this, our first letter, from a communicant in Istanbul (name on request). It is here reproduced, unchanged and in its entirety. *Ed.*]

Dear Sir:

In this week we find a new discovery, a new disease, and we want to inform the doctors by your medical publications. Then please send to our adress one number of that publication in the future to see our writing.

* * *

The disease:

The sick person is a boy, his age is (15) years.

The symptomes:

Genel weakness, sever anemia, pale skin, scratching inter the nose and very chronic hemorrhage of the nose (epistaxis).

All of antihemorrhage, coaggulan and other medicines cannot make any influence and the hemorrhage is continuous.

The blood dont stop with any thing.

In the analysing of the blood we see that the blood and its coaggulation is very normal, and there are no any anormality in the blood.

Then we give anodyne drugs against the scratching, but there are no influence.

In the inspection of the nose we dont find any anormality, but when we pay attention to look inside the nose we see in the deep part of the nose a very small pale spot, this spot sometimes moves to the left and right. We catch it by the tweezers and pull it a little to the outside. When we are pulling it, it dont make any pain, and we see that this pale spot is a head of a long and elastic body, and this body graduaLy becomes long and long, and be more thin.

In the first time we think that it is like the vein or arter, and for this reason we let it to go to its place to the deep of nose.

The blood continuous fulls down.

In the second time we catch it and pull to outside slowly, its length became nearly (15) cm. and it became very thin like the hair, and then it be torn.

It is a "leech".

Now the hemorrhage, exactly after pulling the leech, instantly stoped, there are no hemorrhage at all.

The reason of the hemorrhage is from the anticoagulation ferment of the leech.

The sick person only after one week (5-6) days becomes well.

"End"

Announcement

The inauguration of the A. Graeme Mitchell Memorial Lectureship will take place Feb. 22, 1961, at the Cincinnati Children's Hospital. Dr. Mitchell was professor of pediatrics at the University of Cincinnati College of Medicine from 1924 to 1941. The inaugural program will be a "Symposium on Adolescence," with participation by Drs. Waldo E. Nelson, Joseph Johnston, J. Roswell Gallagher, Thomas Cone, Richard E. Wolf, and Thomas Shaffer and Judge Benjamin Schwartz.

The afternoon symposium will be followed by a dedication banquet and a program in the evening devoted to "Civic Programs for the Care of the Adolescent Child." Guests are welcome, and reservations may be made by writing to Dr. Carl Ochs, 2727 Madison Rd., Cincinnati 9, Ohio.

Some useful principles and practices for modern drug therapy

The development of many new, potent, and highly effective drugs for use in drug therapy has in itself created a problem for the physician. Prior to 1935 there were relatively few potent, highly useful agents available for use in therapy. The physician soon became familiar with these few and was able to use them wisely and with considerable skill. Consequently, there were comparatively few iatrogenic reactions from drug therapy. Furthermore, physicians were so thoroughly acquainted with these drugs that they were able to secure the maximum benefit from them with a minimum of untoward effect.

In the past two decades there has occurred a terrific resurgence in therapy. This acceleration has led to the development of approximately thirty to forty new therapeutically effective chemical entities each year. The physician now has many new drugs, some with complex actions, some with wide or unusual variations in dosage, others with similar action but different chemical or pharmacologic properties, still others with toxic effects that are unusual or difficult to prevent or avoid. He not infrequently finds himself using new drugs which are not well known to him and with which he has no background of experience or any appreciable degree of skill in handling. This can and does lead to iatrogenic reactions, but perhaps even more unfortunate is the inability to use the drugs to secure the maximum benefit in the treatment of his patients.

There is every indication that an ever increasing stream of new agents will continue to appear; therefore, it is necessary for the physician to make every effort possible to handle them wisely. It is not enough for him to blindly follow the brief instructions of the manufacturer which unfortunately in the case of some new agents often comprise about the only information readily available. As a physician he must never delegate his responsibility for his patient to the written instructions of a drug circular. Therefore before using a new drug he should insist upon and become thoroughly familiar with certain essential information the knowledge of which will enable him to secure the maximum benefits with the minimum degree of untoward effects.

There are certain principles and practices which when recognized and followed intelligently will often enable the physician to accomplish the most from the use of drugs. These general rules for good drug therapy will be considered under the following headings: dose, actions other than the principle or desired pharmacologic effect, absorption, duration of action, elimination, action on metabolic processes, influence on enzymatic action, interference with organ function, action on specific tissue or tissue sites, and finally allergy.

Dose

It would seem that there should be no problem concerning the dose of a drug by the time it is released for general use. Unfortunately, however, dose does and will continue to present a serious problem in the use of drugs. It must always be individualized to a particular patient at the particular time in his illness. This principle is often neglected or widely abused, with the result that the drug either is not giving the maximum therapeutic effect it is capable of or

is producing a toxic effect. Almost every physician now knows how to titrate the dose of digitalis to the needs of his patient. The wise physician still follows the precepts laid down by William Withering in his book long ago:

"Let the medicine therefore be given in the doses, and at the intervals mentioned above:—let it be continued until it either acts on the kidneys, stomach, the pulse or the bowels; let it be stopped upon the first appearance of any one of these effects, and I will maintain that the patient will not suffer from its exhibition, nor the practitioner be disappointed in any reasonable expectation."

The essential message contained in this pharmacologic classic should be applied to all drug therapy. That physicians have not done so is readily evidenced by the hundreds of mixtures in common use for many of which it would be impossible to properly titrate the dose of any one agent, let alone all the ingredients, to the patient's individual needs at the particular time in his illness.

It is evident then that the first principle in proper drug therapy is to titrate the dose to the individual and his needs. To blindly follow instructions on a circular is to court difficulties. An example witnessed not long ago illustrates this point. The recommended dose of tolbutamide (Orinase) on the drug circulars at the time was 3 Gm. the first day, 2 Gm. the second day, and 1 Gm. the third day, with the maintenance dose adjusted as needed. A young physician inexperienced in the use of the drug followed these instructions, and a dose of 3 Gm. of tolbutamide was given to an elderly, feeble, malnourished, mildly diabetic female. Later in the day the patient went into profound shock and nearly died before it was discovered that the blood sugar had fallen to a dangerously low level. At least one death from this type of a drug reaction has occurred.[6]

Pharmacologic actions other than the principle or desired effect

Very few drugs are so specific in action as to be entirely devoid of all effects but the desired one. Before a drug can be administered wisely, these other actions should be known and their effect taken into consideration. Not all secondary effects are undesirable, and when taken into consideration they can be useful as well as harmful. For example, the antihistamine diphenhydramine (Benadryl) exerts a rather prominent sedative effect in most patients which may, in certain patients, make it a dangerous drug to use when they are about their daytime activities, but at night its sedative properties will often make it the drug of choice. Unfortunately, the side effects of certain drugs are not readily apparent and may be so subtle as to be ignored by the unwary physician. An example of this are the many antiparkinsonism agents and their tendency to produce glaucoma. The physician maintaining patients on these drugs, as well as on the bowel antispasmodics, must check his patients' eyes for glaucoma if tragic consequences are to be avoided in the susceptible individuals.

Absorption

It is necessary, for intelligent drug use, to know the speed and to what degree a drug is absorbed from the gastrointestinal tract. Drugs that are rapidly and completely absorbed are, in general, more predictable in behavior, and the dose can frequently be more readily and accurately arrived at. Those that have a delayed or incomplete absorption as well as those that must be given in large amounts to get sufficient absorption are usually more erratic in behavior. They are more apt to be influenced by changes in bowel status, and frequently it is

more difficult to arrive at the most effective dose. These factors in the case of drugs with a narrow therapeutic index can make it difficult to secure the maximum therapeutic benefit without getting toxic effects.

Duration of action

Drugs can be divided into four categories on the basis of their duration of action: ultra short, short, intermediate, and long acting. This classification has been applied to the sedatives but can be applied to other drugs as well. In general, those agents which act a few minutes belong to the first, those which act longer than 1 hour but less than 4 to the second, those which act 4 to 12 hours to the third, and drugs which exert an effect from 12 to 14 hours or longer to the fourth category.

The physician needs to know the duration of action in order to intelligently adjust the dose pattern. Furthermore, in the case of drugs with long duration of action, he must be alert to avoid accumulation and subsequent toxic effects. Drugs with long duration of action are more likely to accumulate in the debilitated, those with lowered metabolic activity, the aged with their reduced, cardiac, renal, and perhaps liver reserve, and those who as a consequence of their disease may have impaired ability to handle any agent. In these patients, intermediate acting drugs may become long acting; the dose of the longer acting drugs should be reduced, and shorter acting drugs should be substituted where possible.

Elimination

The mechanism or route of dissipation or removal of a drug is intimately connected with its role in the organism, and no drug can be utilized intelligently unless this information is available. Obviously, a drug removed by the kidneys must be handled differently in those patients with less renal reserve or definite renal impairment from one dependent on the liver for its removal. Furthermore, the speed of elimination is important. Drugs slowly excreted tend to accumulate and lead to toxic effects, while those very rapidly eliminated may be erratic in behavior. For example, barbital is a long acting, slowly eliminated drug dependent on renal excretion. While normally a nontoxic drug, it can be toxic when given to patients with impaired renal function or to the elderly with sufficient impairment of renal reserve to lead to accumulation. Likewise, streptomycin excreted by the kidney is in the usual doses of no great concern but in the presence of reduced renal reserve, whether resulting from disease or age, can readily cause serious vestibular toxicity. In general, patients on slowly eliminated drugs must be carefully followed if the drug is given over long periods of time and if toxic effects are to be avoided.

Action on metabolic processes

As we gain more information about drug action, it becomes more apparent that many drugs profoundly influence metabolic processes. Sometimes this is beneficial, other times detrimental, to the organism. Sometimes these effects are brought about by interference of the drug with absorption of an essential dietary item such as mineral oil in large amounts, leading to a loss of vitamins A, D, and K. Another situation is the removal or exhaustion of an essential metabolic agent by the continued administration of a drug. Examples of this type of effect

are many, but two will suffice: (1) the loss of pyridoxine leading to peripheral neuritis when large and long-continued doses of isoniazid are given, because pyridoxine is conjugated with a metabolite of isoniazid[1] and (2) the development of a macrocytic anemia from prolonged administration of diphenylhydantoin (Dilantin), primidone (Mysoline), and more rarely amobarbital, phenobarbital, or secobarbital. Apparently these drugs exhaust or interfere with the normal metabolism of folic acid.[4]

Unquestionably, certain other serious drug reactions probably will be ultimately explained by similar findings. It is therefore necessary for the physician to be alert for these reactions when any drug is long continued. The more they are searched for, the more we will find.

Influence on enzymatic action

Many drugs exert their effect by influencing enzymatic action. Neostigmine (Prostigmin) is a classic example of this type of action. The monoamine oxidase inhibitors likewise may exert certain aspects of their action because of their effect upon monoamine oxidase. It is also possible that, as in the case of neostigmine, they too can cause toxic effects by virtue of their action on the specific enzyme.

If the enzymatic systems are normal, the individual may react in a much different way to a drug than he would if a single or several enzymatic systems were abnormal. Often it is impossible to know when there is an abnormality. The severe reaction experienced in patients receiving succinylcholine who have low levels of pseudocholinesterase is an example. This situation apparently occurs in about 1 person in 2,800, and there is no way to differentiate these individuals from normal persons without first measuring their pseudocholinesterase levels.[5]

More readily understood or recognized are the premature infants who have poorly developed enzymatic detoxification mechanisms. Premature infants cannot utilize efficiently the carbonic anhydrase, glucuronyl transferase, methemoglobin-reductase, and tyrosine-oxidizing enzyme systems; therefore, they are prone to get serious toxic effects when given drugs requiring detoxification or action by any of these systems. Examples of such drugs are chloramphenicol, nitrites, phenacetin, and other aniline dyes, vitamin K, and the sulfonamides.[2, 3]

Any drug given to a premature infant, therefore, must be carefully administered and reduced in dosage. This is particularly so if it is detoxified by glycuronic acid conjugation or acetylation. Any drug likely to throw a heavy load on the liver should be used with care in these children, since other, as yet unrecognized defects may be present.

Interference with organ function

Many drugs in one way or another interfere with the normal functions of the brain, heart, lungs, kidney, liver, and bone marrow, as well as special organs, such as the ear, eye, and vestibular apparatus. Drug action on the brain can cause excitement and agitation such as occurs with the amphetamines. Disorientation, confusion, and even hallucination can be precipitated by atropine and the antispasmodic and antiparkinsonism drugs in certain patients. Usually, these reactions are seen in the elderly and more particularly the senile person with arteriosclerosis. It is as if the brain in these patients did not have sufficient

reserve to meet the added stress of the drug. Certainly, any new drug of these series given to such patients should be administered with care.

Cardiac arrhythmias may result from the action of many drugs. The antihistamines, the amphetamines, atropine, meperidine, neostigmine, reserpine, and overdosage of the cardiac glycosides all can and do influence heart action. It is important to know and look for such with a new drug of any of the above series.

There are many drugs which affect one organ or another, and as new drugs appear, undoubtedly some will affect not only the brain, heart, kidney, liver, and bone marrow, but other organs as well. The more alert we are, the quicker they will be recognized.

Drugs that affect specific tissues

The list of agents that affect specific tissue sites is growing. Actions of streptomycin on the vestibular apparatus, dihydrostreptomycin on the auditory nerve, and stilbamidine on the carbonic anhydrase inhibitors are now well recognized. Newer agents in these classes have been shown to exert similar effects. Therefore, selective tissue effects must be looked for when using any new drug, and most particularly if drugs of the above series are employed.

Allergy

Finally, a few words need to be said about allergy. This factor is and should be in every physician's mind whenever he prescribes a drug. The family background and the individual's own allergic history must be thoroughly explored before a drug is prescribed. Those with a serious allergic background should be watched carefully. Smaller and, when in doubt, careful trial doses should be given, and the oral route should be used whenever possible.

Although there are many exceptions, in general it is wiser to use those agents with which the desired therapeutic effect is produced by introducing the least amount of a foreign chemical into the body. Many believe less allergy results and less serious reactions occur if the total amount of the introduced new chemical is small. There are many examples which indicate that this is likely to be the case. Two examples are well recognized. When a small amount of horse serum is given prophylactically, a certain small percentage of the population will get a serum sickness reaction; however, if a large dose is given approximately 80 per cent of the population will get the reaction. The same seems to hold for drugs such as penicillin, with which the allergic reaction rate is about 0.6 per cent and fairly constant when the daily dose is 6 million or less, but with which the incidence of allergy rapidly rises to about 8 per cent when the daily amount exceeds this level. Therefore, it is probably wise to select and use those drugs of a series which are the most potent and, consequently, lead to the introduction of the smallest amount of the foreign molecule.

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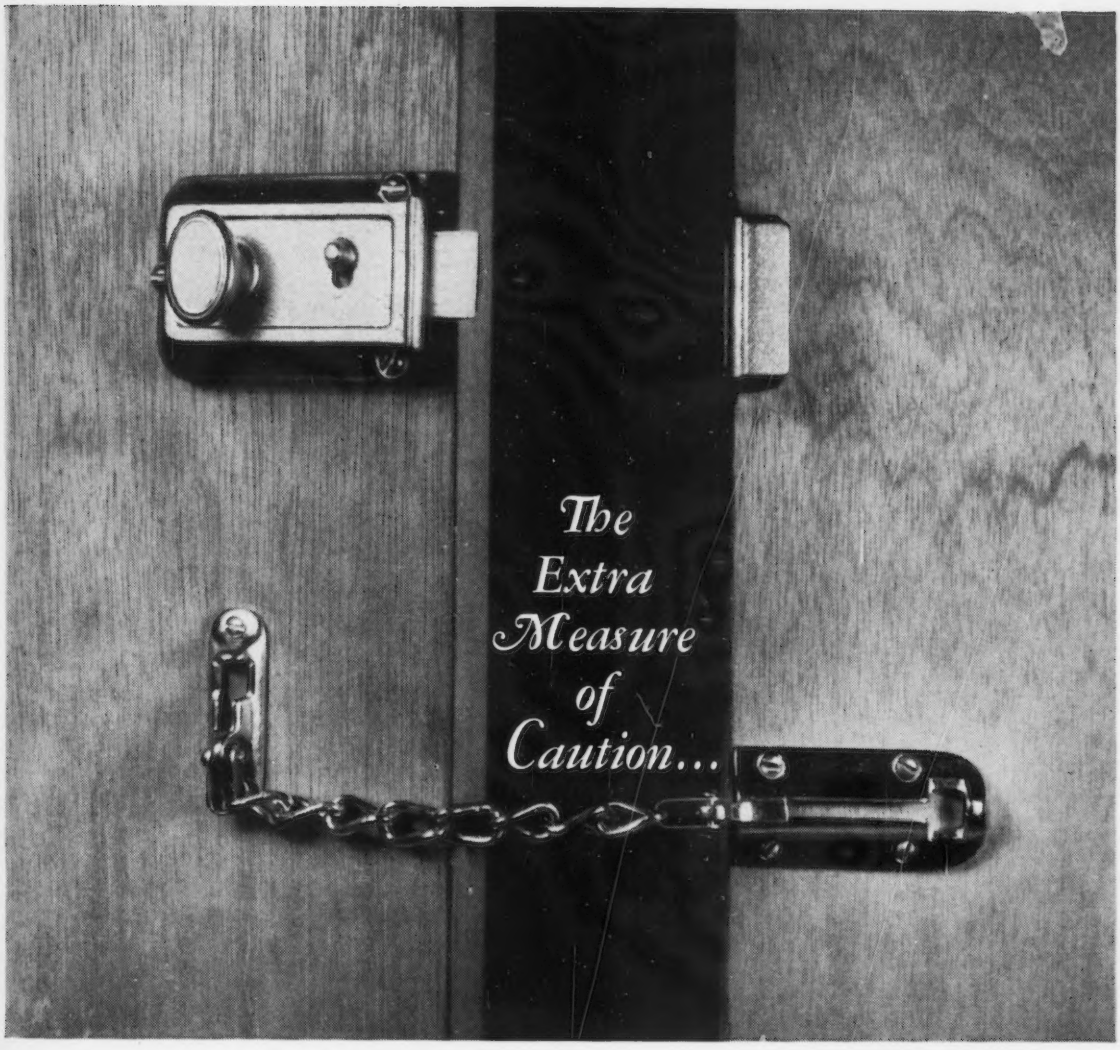

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